DENDRITIC CELL AND MACROPHAGE NOMENCLATURE AND CLASSIFICATION

EDITED BY: Florent Ginhoux, Martin Guilliams and Shalin Naik

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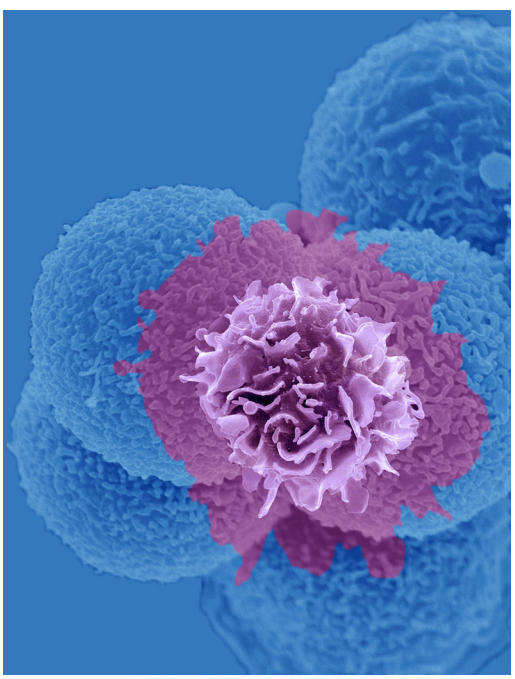
DENDRITIC CELL AND MACROPHAGE NOMENCLATURE AND CLASSIFICATION

Topic Editors:
Florent Ginhoux, Singapore Immunology Network (SIgN), A*STAR, Singapore
Martin Guilliams, Ghent University, Belgium
Shalin Naik, The University of Melbourne, Australia



The blue background represents a cluster of human plasmacytoid dendritic cells, coloured scanning electron micrograph (SEM), and the purple foreground represents a myeloid CD1c dendritic cell.

Image by Benoit Malleret.

The mononuclear phagocyte system (MPS) comprises dendritic cells (DCs), monocytes and macrophages (MØs) that together play crucial roles in tissue immunity and homeostasis, but also contribute to a broad spectrum of pathologies. They are thus attractive therapeutic targets for immune therapy. However, the distinction between DCs, monocytes and MØ subpopulations has been a matter of controversy and the current nomenclature has been a confounding factor.

DCs are remarkably heterogeneous and consist of multiple subsets traditionally defined by their expression of various surface markers. While markers are important to define various populations of the MPS, they do not specifically define the intrinsic nature of a cell population and do not always segregate a bona fide cell type of relative homogeneity. Markers are redundant, or simply define distinct activation states within one subset rather than independent subpopulations. One example are the steady-state CD11b+ DCs which are often not distinguished from monocytes, monocyte-derived cells, and

macrophages due to their overlapping phenotype. Lastly, monocyte fate during inflammation results in cells bearing the phenotypic and functional features of both DCs and MØs significantly adding to the confusion. In fact, depending on the context of the study and the focus of the laboratory, a monocyte-derived cell will be either be called "monocyte-derived DCs" or "macrophages". Because the names we give to cells are often associated with a functional connotation, this is much more than simple semantics. The "name" we give to a population fundamentally changes the perception of its biology and can impact on research design and interpretation.

Recent evidence in the ontogeny and transcriptional regulation of DCs and MØs, combined with the identification of DC- and MØ-specific markers has dramatically changed our understanding of their interrelationship in the steady state and inflammation. In steady state, DCs are constantly replaced by circulating blood precursors that arise from committed progenitors in the bone marrow. Similarly, some MØ populations are also constantly replaced by circulating blood monocytes. However, others tissue MØs are derived from embryonic precursors, are seeded before birth and maintain themselves in adults by self-renewal. In inflammation, such differentiation pathways are fundamentally changed and unique monocyte-derived inflammatory cells are generated.

Current DC, monocyte and MØ nomenclature does not take into account these new developments and as a consequence is quite confusing. We believe that the field is in need of a fresh view on this topic as well as an upfront debate on DC and MØ nomenclature. Our aim is to bring expert junior and senior scientists to revisit this topic in light of these recent developments. This Research Topic will cover all aspects of DC, monocyte and MØ biology including development, transcriptional regulation, functional specializations, in lymphoid and non-lymphoid tissues, and in both human and mouse models. Given the central position of DCs, monocytes and MØs in tissue homeostasis, immunity and disease, this topic should be of interest to a large spectrum of the biomedical community.

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EDITORIAL
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Editorial: Dendritic Cell and Macrophage Nomenclature and Classification

Florent Ginhoux[1†], Martin Guilliams[2,3*†] and Shalin H. Naik[4,5*†]*

[1]Singapore Immunology Network (SIgN), A*STAR, Singapore, [2]Laboratory of Immunoregulation, VIB Inflammation Research Center, Ghent University, Ghent, Belgium, [3]Department of Respiratory Medicine, Ghent University, Ghent, Belgium, [4]Molecular Medicine Division, The Walter + Eliza Hall Institute of Medical Research, The University of Melbourne, Melbourne, VIC, Australia, [5]Department of Medical Biology, The University of Melbourne, Melbourne, VIC, Australia

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***Correspondence:**
Florent Ginhoux
florent_ginhoux@immunol.a-star.edu.sg;
Martin Guilliams
martin.guilliams@irc.ugent.be;
Shalin H. Naik
naik.s@wehi.edu.au

[†]Florent Ginhoux, Martin Guilliams, and Shalin H. Naik contributed equally to the manuscript.

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The Editorial on the Research Topic

Dendritic Cell and Macrophage nomenclature and classification

Mononuclear phagocytes that include dendritic cells (DCs), monocytes, and macrophages constitute a group of cell types crucial for the control of pathogens and induction of immune responses as well as for the support of tissue functions. These properties make them highly relevant targets for immune therapy, vaccination, and treatment of autoimmune and inflammatory diseases (1, 2). However, exactly how many cell types exist in the mononuclear phagocyte system (MPS), or whether they even combine to constitute a family, has been a matter of contention for many years. Historically, cells of the MPS have, at one time or another, been referred to as erythrophagocytes, pyrrhol cells, adventitia cells, rhagiocrine cells, polyblasts, clasmatocytes, and histiocytes (Yona and Gordon) prior to their current terminology established in 1972 (3). The seminal discovery of a new cell type termed DCs in the 1970s by the late Ralph Steinman that were distinct from macrophages added even more complexity in the MPS classification (4). However, some time passed before DCs were fully accepted as true member of the MPS. Over time, there was appreciation that there were not just one but multiple DC subtypes, each with a specialized role (5). So, while a "*dendritic-shaped cell that can process and present antigen to activate naive T cells*" was a good initial working definition (6), it did not take into account the inconsistent observations that other cells can be dendritic in appearance or activate naive T cells, and that not all "DCs" are immunostimulatory nor dendritic (7). As a result, many different cell types have been given a DC moniker over the years, such as monocyte-derived DCs, conventional DCs (cDCs), and plasmacytoid DCs (8). This appreciation of multiple subtypes has both clarified and confused the field. Importantly, we do not consider the classification and nomenclature issues as trivial semantics. Indeed, classification is of very practical importance in allowing researchers to work to a common framework as highlighted by Norma Lang "If we cannot name it, we cannot control it, finance it, teach it, research it, or put into public policy (page 109)" (9).

The idea behind this Frontiers Research Topic on "Dendritic Cell and Macrophage Nomenclature and Classification" was to have an open debate on the advantages and disadvantages of different classification systems of cells within the MPS. In this Research Topic, 17 contributions from international experts cover the complexity of the MPS, from its ontogeny and transcriptional regulation, its classification in different tissues and different species.

First, in a historical perspective, Yona and Gordon examine the early origins and development of macrophage research from Ilya Metchnikoff's discovery to the establishment of the MPS nomenclature half a century ago.

In an opinion article, Vremec and Shortman discuss issues of DC subset definition encountered in their past work.

In a hypothesis and theory article, Guilliams and van de Laar discuss the practical application of our recently proposed classification system based on ontogeny (8).

Hoeffel and Ginhoux cover the ontogeny of tissue-resident macrophages and discuss evidence suggesting that hematopoietic stem cell-independent embryonic precursors transiently present in the yolk sac and the fetal liver give rise to long-lasting self-renewing macrophage populations.

Tussiwand and Gautier discuss the developmental pathways of murine MPS cells, with a particular emphasis on the transcriptional factors that regulate their development and function.

Poltorak and Schraml review experimental approaches taken to fate map DCs and discuss how these have shaped our understanding of DC ontogeny and lineage affiliation.

In a perspective article, Gottschalk and Kurts review the complexity of the renal MPS, and how to distinguish DCs and macrophages in the kidney from the nephrologist's point of view.

Gross et al. discuss origins and functions of intestinal DCs and macrophages and their respective subsets, focusing largely on the mouse and cells residing in the lamina propria.

Greter et al. discuss myeloid cells in the brain and the difficulties to delineate resident microglia from infiltrating myeloid cells using currently known markers and the recent advances that have helped to make clear definitions between phenotypically similar, yet functionally diverse myeloid cell types of the brain.

Cassado et al. review the heterogeneity of peritoneal macrophages, which exhibit distinct phenotypes, functions, and origins.

Eckert et al. summarize the multiple roles of macrophages and DCs in chronic liver diseases and outline the currently known marker combinations for the identification of these cell populations for the study of their role in liver immunology.

Moving to human cells, Reynolds and Haniffa review the parallel organization of human and mouse mononuclear phagocyte networks. They also discuss the strategies, power, and utility of comparative biology approaches to integrate recent advances in human and mouse mononuclear phagocyte biology, and its potential to drive forward clinical translation of this knowledge.

In a research article, Vu-Manh et al. extend our knowledge of the homology of the MPS across species through comparative transcriptomics. They present an approach combining refined cell sorting and integrated comparative transcriptomics analyses, which revealed conservation of the mononuclear phagocyte organization across human, mouse, sheep, pigs, and chicken.

In a complementary review, Vu-Manh et al. discuss the highly significant conservation during evolution of DC subsets cell surface phenotyping, expression analysis of hallmark genes, and functions.

Ziegler-Heitbrock reviews human blood monocyte heterogeneity and their subdivision into classical, intermediate, and non-classical monocytes, and how these proportions change during inflammation and discuss its relevance to management of disease.

Durand and Segura review recent advances in our understanding of the human DC network and discuss some remaining gaps and future challenges of the human DC field.

Finally, in an original research article, Sudan et al. identify novel markers of activated human macrophages through the analysis of gene-expression profiles for human macrophages of a single donor subjected to 33 distinct activating conditions.

Altogether, the many contributions to this Frontiers Research Topic not only underline the complexity of the MPS system but also highlight the similarities between MPS cells of different tissues. Moreover, classifying MPS cells based on their gene-expression profiles results in a classification system that is close to the classification of cells based on their cellular origin and development. Although a final basis for MPS classification has not been defined yet, we hope this Frontiers Research Topic will pave the way toward a wider consensus within the field.

REFERENCES

1. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol* (2014) **14**:392–404. doi:10.1038/nri3671
2. Schlitzer A, Ginhoux F. Organization of the mouse and human DC network. *Curr Opin Immunol* (2014) **26**:90–9. doi:10.1016/j.coi.2013.11.002
3. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ* (1972) **46**:845–52.
4. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* (1973) **137**:1142–62. doi:10.1084/jem.137.5.1142
5. Schlitzer A, McGovern N, Ginhoux F. Dendritic cells and monocyte-derived cells: two complementary and integrated functional systems. *Semin Cell Dev Biol* (2015) **41**:9–22. doi:10.1016/j.semcdb.2015.03.011
6. Naik SH. Demystifying the development of dendritic cell subtypes, a little. *Immunol Cell Biol* (2008) **86**:439–52. doi:10.1038/icb.2008.28
7. Perié L, Naik SH. Toward defining a "lineage" – the case for dendritic cells. *Semin Cell Dev Biol* (2015) **41**:3–8. doi:10.1016/j.semcdb.2015.02.004
8. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**:571–8. doi:10.1038/nri3712
9. Clark J, Lang N. Nursing's next advance: an internal classification for nursing practice. *Int Nurs Rev* (1992) **39**:109–28.

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REVIEW
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From the reticuloendothelial to mononuclear phagocyte system – the unaccounted years

Simon Yona[1]* *and Siamon Gordon*[2]

[1] University College London, London, UK, [2] Sir William Dunn School of Pathology, The University of Oxford, Oxford, UK

It is over 125 years since Ilya Metchnikoff described the significance of phagocytosis. In this review, we examine the early origins and development of macrophage research continuing after his death in 1916, through the period of the reticuloendothelial system. Studies on these cells resulted in a substantial literature spanning immunology, hematology, biochemistry, and pathology. Early histological studies on morphology and *in situ* labeling laid the foundations to appreciate the diversity and functional capacity of these cells in the steady state and during pathology. We complete this phagocyte retrospective with the establishment of the mononuclear phagocyte system nomenclature half a century ago.

Keywords: macrophage, monocyte, Metchnikoff, phagocytosis, dendritic cells, inflammation

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Peter M. Van Endert, Université Paris Descartes, France

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Edda Fiebiger, Harvard Medical School, USA
Richard A. Kroczek, Robert Koch-Institute, Germany
Jean M. Davoust, Institut National de la Santé et la Recherche Médicale, France

***Correspondence:**
Simon Yona, University College London, 5 University Street, London WC1E 6JF, UK
s.yona@ucl.ac.uk

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Introduction

The earliest accounts of macrophage research are closely linked with the widespread introduction of the microscope in the mid-nineteenth century, 300 years following the seminal microscopic observations of Antony van Leeuwenhoek (1700) (1). In the histological accounts, von Kölliker (1847) detected cells in the spleen containing particles; later Preyer (1867) observed the internalization of erythrocytes by splenic cells and proposed that this occurred by an active process (2, 3). However, investigators at the time did not associate such observations with a defense mechanism. In fact, Klebs (1872) believed just the opposite, proposing that these cells assist the transport of bacteria to lymphatic tissue (4). Koch (1878) also concluded that these cells provide a suitable microenvironment for bacilli to multiply and disseminate to other tissues, – the so-called Trojan horse theory – after observing numerous bacilli within leukocytes, while studying frogs treated with anthrax (5). Therefore, although cytological observations of the mid-nineteenth century recognized the ability of leukocytes to devour (*fressen*) erythrocytes and microorganisms, opinion at the time did not associate this event with host defense, nor was there a consensus that the process was active or merely the penetration of foreign material into cells to aid infection.

By the late nineteenth century, Metchnikoff (1892), the Russian zoologist and forefather of cellular immunity, established the idea of the phagocyte (6–8). Metchnikoff was the first to fully appreciate the capabilities and purpose of phagocytosis, by performing a series of classical studies spanning from simple unicellular organisms to complex vertebrates. The description of Metchnikoff's discovery of phagocytosis documented by his wife Olga, now rests in the pantheon of immunology legend.

> . . . One day when the family had gone to see some performing apes at the circus, Metchnikoff with his microscope introduced a rose thorn into the transparent body of a starfish larva, Metchnikoff observed the accumulation of phagocytes surrounding the foreign material and attempting to devour the splinter. . . (9).

It is important to remember that Metchnikoff started his career as an evolutionary developmental embryologist, influenced by Darwin's recent publication On the Origin of Species in 1859. As an embryologist, Metchnikoff modeled the early formation of the embryo in primitive organisms, such as sponges, and proposed that an inner "*parenchymella*" contained wandering cells of mesodermal origin capable of taking up particulate matter during embryogenesis. These studies may have been the foundation for Metchnikoff's phagocytosis theory. Later, Metchnikoff recognized the multiple tasks performed by phagocytosis; as an embryologist, the reabsorption of tissue during embryogenesis, as a zoologist, a common feeding mechanism of unicellular organisms and as a pathologist, its role in host defense. Therefore, when Metchnikoff performed his most notable study, the rose thorn experiment at Messina culminated in the phagocytic process we know today. Metchnikoff was one of, if not the, earliest to demonstrate the evolutionary functional adaptation of a particular biological process, in this case phagocytosis, from a simple feeding mechanism for unicellular organisms, to a developmental requirement during embryogenesis and finally as a necessity for host defense (3, 10).

Metchnikoff's phagocytes comprised two populations he termed macrophages (large eaters) and microphages (small eaters, later known as polymorphonuclear leukocytes). Contrary to Rudolf Virchow's impression that inflammation is a continuous life threatening menace, Metchnikoff regarded it as a healing or salutary reaction as postulated 100 years earlier by the Scottish surgeon and collector John Hunter (1794) (11). Therefore, Metchnikoff concluded that the ability of cells to engulf foreign microorganisms acts as an active defense mechanism, giving rise to the concept of cellular innate immunity. At the time, this triggered extensive debate between humoral and cellular schools of thought. Two major events at the turn of the twentieth century helped to reconcile this dispute. First, in 1908, the Nobel Prize in Physiology or Medicine was awarded jointly to Metchnikoff, advocate of the cellularists and to Ehrlich, the champion of humoralist dogmas "*in recognition of their work in immunity*". Second, in 1903, Wright and Douglas proposed the concept of "opsonization" as a humoral mechanism to increase the susceptibility of bacteria to phagocytosis. These investigators claimed that humoral and cellular functions were not mutually exclusive, rather interdependent (12). This theory was spoofed by George Bernard Shaw, in the introduction to his play "The Doctor's Dilemma" in 1906.

> . . . Sir Almroth Wright, following up one of Metchnikoff's most suggestive biological romances, discovered that the white corpuscles or phagocytes, which attack and devour disease germs for us, do their work only when we butter the disease germs appetizingly for them with natural sauce which Sir Almroth Wright named opsonin. . . (13).

The Reticuloendothelial System

By the early decades of the twentieth century descriptions of the phagocyte system had become chaotic, not least since the term macrophage had become synonymous with adventitia cell, anode cell, clasmatocyte, dictocyte, erythrophagocyte, histiocyte, polyblast, pyrrhol cell, and rhagiocrine cell; the many terms bestowed on these cells (>30 different names) (14) revealed that the divergence of opinion at the time as to the relationship of these cells to one another and from tissue-to-tissue. Not only were tissue phagocytes given a variety of bewildering names but also their origin remained unknown. From time-to-time, historic discoveries are lost in the ether of *a priori* thought; this is certainly true for histological techniques that assisted in the classification of blood cytology. Until Ehrlich's early effort to develop leukocyte cytological staining, scholars of blood operated solely on fresh samples. Ehrlich's aim was to take advantage of the known chemical structures of dyes and their interaction with cellular bodies to map and characterize the anatomy of blood cells. By using aniline dyes in combination with neutral dyes and the morphology of the nucleus, he was able to divide cells of the blood into mononucleated lymphocytes, some of which were large, large mononuclears with indented nuclei (now known as monocytes) and polymorphonuclear cells that were neutrophilic (neutrophils), acidophilic (eosinophils), or basophilic granules (basophils/mast cells) (15). By the early twentieth century, Ribbert (1904) had performed studies with lithium carmine solution injected into the peripheral circulation and observed the specific uptake and storage by a group of cells, which became *vitally stained* (16). These were subsequently demonstrated to be mononuclear cells phagocytosing particulate matter. Clark and Clark (17) described these large mononuclear cells in tissues to be the same as "clasmatocytes," described by Louis-Antoine Ranvier, the "Polyblasts" of Alexander Maximow and the "Histiocytes" defined by Kenji Kiyono. Following these early observations, it became apparent that a large number of histological dyes including trypan blue, neutral red, isamine blue, and other colloids discriminated phagocytes from fibroblasts. The systematic analysis of tissues and dyes led Karl Albert Ludwig Aschoff (1924) to coin the term "reticuloendothelial system" (RES) to describe this group of cells, with their ability to incorporate vital dyes from the circulation (18). Reticulo refers to the propensity of these large phagocytic cells in various organs to form a network or a *reticulum* by cytoplasmic extensions; *endothelial* refers to their proximity to the vascular endothelium (19), from which they were sometimes believed to arise, these cells formed Aschoff's unified system throughout the organism. The capture and clearance of unwanted particulate material from blood and lymph were considered to be the major function of the RES. Although opinions about the origin of cells of the RES will be discussed later in this series; at the time, Aschoff considered that the cells of the RES were derived locally and that both histiocytes and reticulum cells shared a common origin.

Cells of the Reticuloendothelial System

Metchnikoff had previously described the dissemination of macrophages throughout the organism and Aschoff's system implied a common function of the cells of the RES even in the absence of inflammation. In the next section, we highlight some of the tissue locations and possible functions assigned at the time.

Kupffer Cells

The macrophages of the liver are located within the sinusoids, which is composed of four cell types, each with its own morphology and function. Karl Wilhelm von Kupffer (1876) observed "*Sternzellen*" (star cells) in the liver and believed them to be an integral part of the hepatic endothelium. Later, Tadeusz Browicz (1899) identified Kupffer's cells as the phagocytes of the liver (20) (sometimes known as Browicz–Kupffer cells) and observed that they could take-up a large percentage of vital stain. In the early 1930s, Peyton Rous developed an ingenious method to isolate Kupffer cells of the liver. Rous and Beard injected a suspension of gamma ferrous oxide i.v., light in weight but highly magnetic particles, Kupffer cells efficiently phagocytosed these particles. They then perfused and processed the liver and the phagocytes were then selected by magnetic force, to the best of our knowledge the first description of magnetic cell sorting (21, 22), enabling the extraction of macrophages from a solid tissue for examination *in vitro*. The origin of Kupffer cells like all cells of the RES at the time remained a source of continued confusion and debate. At the American Association of Anatomists conference in 1925, M. R. Lewis presented a paper comparing Kupffer cells isolated from frogs, thought to be derived from endothelial cells, side-by-side with an examination of clasmatocytes and concluded these cells were identical in morphology and function (23).

In 1950s, Baruch Benacerraf, Nobel laureate in 1980 for his work on MHC with George Snell and Jean Dausset (24), teamed up with Guido Biozzi, a young Italian in the Halpern laboratory in Paris in a productive collaboration. They developed techniques to study clearance of particulates from blood and formulated equations that govern this in mammals. In subsequent work, Biozzi bred strains of mice differing in the quantitative antibody response to various antigens. Biozzi mice are still in use to study autoimmune inflammatory neurological disease (25). These studies in mice and guinea pigs helped to introduce genetic approaches to the role of macrophages in innate and adaptive immunity.

Microglia and the Origin of the RES

Virchow (1858) acknowledged that the central nervous system (CNS) was composed of both neurones and interstitial cells, which he termed neuroglia (26, 27). By the end of the nineteenth century, the Scottish pathologist William Ford Robertson confirmed that neuroglia were indeed composed of multiple cell types (28). Robertson continued to investigate this heterogeneous population of cells; with the aid of platinum staining techniques he was able to distinguish a novel cell type in the brain he termed mesoglia (as he believed that they were mesodermal in origin). Finally, Robertson deduced that mesoglia possessed phagocytic properties (29). In fact, Robertson had identified oligodendroglia and mesodermal derived cells, under the term mesoglia. In 1913, Santiago Ramon y Cajal described a group of cells derived from the mesoderm as the "*third element*" of the CNS, the first element being neurones and the second element the astrocytes, derived from ectoderm. It was the Spanish pathologist Pio Del Rio-Hortega who revolutionized our understanding of neuroglia from a series of detailed studies using silver carbonate impregnation staining. He uncovered a homogeneous group of cells within the CNS with tree like projections and predicted that they possess phagocytic functions within the CNS; he termed these cells as microglia (26, 27). Hortega laid the groundwork for microglia research in a lecture given at University of Oxford, microglia enter the CNS during development from mesodermal origin where they disseminate throughout the CNS and take-up a branched ramified cytological appearance. He went on to explain that they remain evenly spaced in the steady state, while pathological insults cause microglia to take on an amoeboid morphology, express the ability to phagocytose and to migrate (30). These studies confirmed that the microglia of the CNS belonged to the RES. The account he gave in Oxford remains accurate to this day. Interestingly, although microglia were unable to take-up vital dyes because of the blood brain barrier, they were known to be highly phagocytic, reticuloendothelial cells readily stained by silver carbonate. Although Hortega described microglia to be derived from cells of the mesoderm during embryogenesis, this still remained a matter of great debate, until recently. Early observations in the late nineteenth and several studies in the early twentieth described microglia during neurodegenerative diseases without a clear understanding of their origin.

Osteoclasts

The histological identification of a cell that resorbs bone can be traced back to the early 1850s. Tomes and De Morgan (31) described within a section of diseased femur, cavities that were associated with an increase in nucleated cells (31). By 1873, Kölliker described multinucleated giant cells involved in bone absorption that he termed *Ostoklast* and anticipated that these cells are involved in homeostatic and pathological bone degradation (32, 33). The notion of a bone-resorbing cell became widely accepted (34). Over the next 50 years, the morphology of the osteoclast was refined and interestingly these large multinucleated cells showed variation in size and nuclear content; in pigs, they contained as many as 100 nuclei (35) while human osteoclasts could contain up to 50 nuclei (32). John Loutit an Australian born pioneer in radiation biology from the late 1940s studied not only osteoclast origin from blood precursors but also the biology of bone marrow transplantation after irradiation (36) in a long and productive career at Harwell MRC laboratories.

Alveolar Macrophages and Phagocytosis

The lung also contains many mononuclear phagocytes, which are associated with the alveoli and the alveolar space (37). The macrophages within the alveolar space were initially known as "dust cells" because of their content of intracellular carbon particles. There is a constant requirement to keep the alveolar space free of particles and pathogens allowing for optimal oxygen transfer, the major role of these cells. As the lung occupies a unique accessible position among internal organs, it is constantly in contact with the external world. In the late 1950s, Karrer observed the efficient phagocytosis of India ink exclusively by free alveolar macrophages, similar to the previous observations of increased

carbon particles in these cells of city dwellers (38, 39). The question of the origin of the macrophage troubled cytologists and immunologists for most of the twentieth century; this was no different in the lung.

One of the best-studied pathologies in the first half of the twentieth century in relation to macrophages was pulmonary tuberculosis. The initial stage of tuberculosis displays the transient influx of neutrophils described by Maximow in 1925 (40). However, these cells are unable to destroy the bacilli and monocyte/macrophages remain the most prominent infected host cells. From 1920s, Sabin, the first full female Member of the Rockefeller Institute and first female elected to the National Academy of Sciences, considered the monocyte response to tuberculosis the most significant process, "*cellular and immunological reactions in tuberculosis center around the monocyte*," when she first proposed this she was mocked by her peers. Sabin observed monocytes to become epithelioid cells that develop into macrophage giant cells (41), previously described by the German pathologist Theodor Langhans as a hallmark of tuberculous granulomata already in the nineteenth century. In 1930s, Max Lurie, an advocate of the monocyte theory, used inbred rabbits to study their susceptibility to bovine tuberculosis. Lurie observed resistant inbred rabbits went on to develop cavitary tuberculosis while susceptible families went on to develop disseminated tuberculosis (42). The Australian immunologist, George Mackaness studied the role of anti-TB drugs on infected macrophages when a student at the Sir William Dunn School of Pathology, University of Oxford with Howard Florey in the early 1950s. His subsequent studies in the sixties at the Trudeau Institute in Saranac Lake defined T lymphocyte-dependent activation of macrophages by BCG and *Listeria* infection (43, 44), Mackaness coined the term macrophage activation, the so-called "angry" macrophages (45). Dannenberg has been another pioneer of macrophage research in experimental and clinical tuberculosis, especially in the characterization of the granuloma (46).

Other resident phagocytic populations were described in many tissues during this period, for example, in the skin (Langerhans cells), gut, lympho-hemopoietic tissues, reproductive and endocrine organs, and placenta (Hoffbauer cells). We draw attention to specialized macrophages in bone marrow stroma, where they appear at the center of hematopoietic islands, first described in detail by Marcel Bessis and his collaborators (47). John Humphrey drew attention to the marginal metallophilic macrophages located in a zone between the red and white pulp of spleen, especially in rodents (48, 49). They line a sinusoidal space where they sample circulating blood for viruses, for example, and play an important role in clearance of T cell-independent immunogenic polysaccharides. Tingible body macrophages (TBM) were identified by Walther Flemming in 1885; located in germinal centers. TBM contain phagocytosed apoptotic cell debris (tingible bodies) and are involved in the clearance of apoptotic lymphocytes (50), these observations were confirmed by electron microscopy in the early 1960s (51). Finally, the peritoneal macrophages of the mouse, responsible for much of our knowledge of macrophage immunobiology, were first described as a tractable cell population by Cohn only in 1962 (52).

The Origin of Macrophages

As Aschoff was formulating the requirements of the RES, a number of research groups were searching for the origin of macrophages. In 1914, Awrorow and Timofejewskij concluded from the outgrowth of leukocytes from leukemic blood that the lymphocyte is the progenitor from which macrophages arise (53). A few years later, several *in vitro* studies described the differentiation of blood monocytes into macrophages (53–55); Carrel and Ebeling (55) and Lewis and Lewis (23) observed that blood cultures over time developed into macrophages that had phagocytosed the debris of other blood cells, concluding that these monocyte-derived cells became actively phagocytic and were indistinguishable from macrophages in staining with neutral red (54, 55). At the same time, in 1925, Sabin took a cytological approach using neutral red staining to examine resident macrophage populations in connective tissue (clasmatocytes), concluding that a proportion of macrophages were derived from bone marrow-derived monocytes (56). However, the first *in vivo* study to examine how mammalian blood monocytes behave during an acute pathological insult was performed by Ebert and Florey (57) at the University of Oxford, using the rabbit ear chamber, observing diapedesis of blood monocytes toward the site of tissue injury. These monocytes transformed into macrophages during the inflammatory process; they concluded "*The cells originating from monocytes eventually became cells which we are calling histiocytes, which are indistinguishable from the so-called resting wandering tissue-cell of Maximow*" (57). Twenty-five years later, Volkman and Gowans (58) confirmed these findings using thymidine autoradiography and parabiosis inferring that macrophage precursors are rapidly dividing cells derived from a remote site during inflammation (58). Takahashi mentions in his comprehensive review on macrophage ontology how the Japanese pathologist Amano with the aid of supravital staining at the inflammatory foci observed blood monocytes to be precursors of the macrophage (59). Finally, Marchesi and Florey employing electron microscopy were able to distinguish the earliest phase of monocyte migration during mild inflammation, which occurred during the maximal efflux of neutrophils (14, 60). The conclusion from these studies suggests that macrophages derived from circulating monocytes were based on inflammatory models. Therefore, a more accurate conclusion would be that during inflammation monocytes become effector cells by concentrating at the site of injury with the ability to produce large quantities of inflammatory mediators (61).

Another important and well-studied population of recruited monocyte-derived cells are the foam cells, a hallmark of atheromatous pathology. A pupil of Maximow in St. Petersburg and later the student of Aschoff in Freiburg (62), Anitschkow (63, 64) showed that simply by feeding rabbits purified cholesterol caused vascular changes leading to the formation of lesions similar to those seen during atherosclerosis in humans (63, 64). Anitschkow decided that these cholesterol-laden cells were of leukocyte origin (65). Anitschkow's work on lipid storage was compared to the work by Robert Koch on the tubercle bacillus (66). It was mainly as a result of the work by Russell Ross in 1970s that these monocyte-derived cells have been categorized

as part of a fat modified inflammatory process (67). Recruited monocytes can also give rise to multinucleated giant cells, not only a feature of tuberculosis. They are found in many granulomatous inflammatory diseases, including viral and parasitic infections, and in responses to foreign bodies and fat necrosis (Touton cells), named after the German dermatologist Karl Touton (1885) (68).

The accumulation of data and the introduction of new techniques highlighted that the cells of the RES differ in morphology, function, and origin (14). In addition, the underlying processes involved in these functional and morphologic alterations remained unknown. Is there a proliferating mononuclear phagocyte population within the RES, constantly differentiating in the steady state? These questions continued to puzzle scientists throughout the twentieth century.

The Mononuclear Phagocyte System

> . . . The most immature cell of the mononuclear phagocyte system . . . is the promonocyte . . . that by dividing gives rise to monocytes . . . Monocytes in the circulation constitute a mobile pool of relatively immature cells on their way from the place of origin to the tissues. At sites where conditions are favourable for phagocytosis, these cells become macrophages. . . (69).

As knowledge accumulated, the term RES was regarded as insufficient to describe resident phagocytes and their antecedents. At a scientific meeting in Leiden in 1969, a group of prominent pathologists/immunologists proposed the term "mononuclear phagocyte system" (MPS) as a more accurate term (**Figure 1**) (69). The MPS at the time comprised monocytes and macrophages derived from the bone marrow derived monocytes. Nevertheless, little evidence existed to suggest that monocytes differentiate into resident macrophages under steady state conditions. Interestingly, Maximow proposed on the basis of embryonic studies on amphibians and mammals that macrophages and leukocytes may arise from distinct lineages (70).

While the MPS was being formulated in 1960s, immunologists were in pursuit of the "*third cell*" (71) a requirement for adaptive-immune responses. Steinman and Cohn in their landmark study (1973) identified and characterized the dendritic cell (DC) as distinct from macrophages (72, 73); over time, the DC became accepted as the third arm in the trinity we know today as the MPS (74). Monocytes, macrophages, and DCs are distinguished on the basis of morphology, function, and origin, yet collectively constitute the MPS.

FIGURE 1 | Selected experimental pathologists and immunologists who coined the term mononuclear phagocyte system.

As more data accumulated in the early decades of the twenty-first century, it emerged that most tissue macrophage populations in adults in the steady state are maintained independent of the bone marrow and rely almost exclusively on self-renewal (75, 59, 76–86). These data facilitated the reexamination of the concept of the MPS (87).

Conclusion

We have highlighted only a few of the many milestones of macrophage biology from its early origins to the establishment of the MPS nomenclature in 1968. Studies during this period resulted in a substantial literature spanning immunology, hematology, and pathology. A number of important issues emerge from a retrospective analysis of the literature. First, we learn that rarely in science do revolutions occur from a single Eureka moment rather years of observation culminate in new findings. While Metchnikoff's phagocytosis theory seems to have emerged from his experiments on starfish larvae, he had previously observed cells capable of taking up particulate matter during embryogenesis (10). Second, the first half of the twentieth century, blighted by two World Wars, had profound impacts on science, resulting in a geographical and common language shift of scientific research. Third, the techniques used routinely in the laboratory shifted from the pathologists' tool box of the microscope and microbiology to the immunologists' introduction of cell transfer, thymidine autoradiography, immunohistochemistry, parabiosis, electron microscopy, later flow cytometry, cell, and molecular biology. However, if one was able to transport Metchnikoff, Aschoff, or Cohn to a conference in 2015 on mononuclear phagocytes they would perhaps not appreciate the cytokines, chemokines, blots, and plots; however, the fundamental questions and discussions remain familiar; what is the origin of these cells? How do they phagocytose? Do macrophages proliferate *in situ*? How is particulate material recognized and cleared? This is why it is important to examine the history of our field since our research questions today are more closely linked to the past than we may appreciate.

The macrophage story is not over. In recent months, further strides have been made in examining the molecular signatures, characterizing the MPS in the steady state and upon enhanced recruitment of monocytes during inflammation (88–94). These studies highlight collective attributes of the macrophages; however, they also show significant local adaptations associated with particular functions within a specific organ. The next stage on this journey will include recreating *in vitro* the phenotypes of these specific populations using induced pluripotent stem cells, and gaining a greater insight into how these cells behave under steady state conditions *in situ*, as well as during and after the inflammatory response. Finally, the role of the circulating monocyte is also undergoing a re-evaluation; previously, monocytes were viewed as the bridge from bone marrow progenitors to fully differentiated tissue macrophages not only after inflammation, injury, and infection but also for resident populations in the

absence of inflammation, as stated in Van Furth's description of the MPS "*Monocytes in the circulation constitute a mobile pool of relatively immature cells on their way from the place of origin to the tissues*" (69). Moreover, monocytes should now be further investigated as distinct precursors of only newly recruited monocyte-derived cells and as effector cells in their own right.

Acknowledgments

We have cited only a fraction of investigations during this period. We dedicate this review to all the scientists who have contributed to the field especially E. Metchnikoff and Z. A. Cohn, whose contributions continue to impact research today.

References

A number of the citations in this review are historical and may be unavailable to readers. If you are interested in obtaining a copy of a particular publication please contact the corresponding author. In addition, please be aware the authors have cited each paper as it appeared at the time of publication, many journal names have changed overtime papers are cited as they appeared at the time of print.

1. Wootton D. *Bad Medicine: Doctors Doing Harm Since Hippocrates*. Oxford: Oxford University Press (2006).
2. Preyer W. Ueber amöboide blutkörperchen. *Arch Pathol Anat Physiol Klin Med* (1867) **30**:417–41.
3. Tauber AI, Chernyak L. *Metchnikoff and the Origins of Immunology: From Metaphor to Theory*. Oxford: Oxford University Press (1991).
4. Klebs E. *Beiträge zur Pathologischen Anatomie der Schusswunden*. Leipzig: von F.C.W. Vogel (1872).
5. Koch R. *Untersuchungen Über Die Aetiologie der Wundinfektionskrankheiten*. Leipzig: von F.C.W. Vogel (1878).
6. Metchnikoff E. *Leçons sur la Pathologie Comparée de L'inflammation Faites à l'Institut Pasteur en Avril et mai 1891*. Paris: G. Masson Libraire de l'academie de Medecine (1892).
7. Metchnikoff E. Ueber den kampf der zellen gegen erysipel-kokken, ein beitrag zur phagocytenlehre. *Arch Pathol Anat Physiol Klin Med* (1887) **107**: 209–49.
8. Gordon S. Elie Metchnikoff: father of natural immunity. *Eur J Immunol* (2008) **38**:3257–64. doi:10.1002/eji.200838855
9. Metchnikoff O. *Life of Elie Metchnikoff 1845-1916*. Boston, NY: Houghton Mifflin (1921).
10. Tauber AI. Ilya Metchnikoff: from evolutionist to immunologist, and back again. In: Harman O, Dietrich MR, editors. *Outsider Scientists Routes to Innovation in Biology*. Chicago, IL: The University of Chicago Press (2013). p. 259–74.
11. Hunter J. *A Treatise on the Blood, Inflammation and Gunshot Wounds*. London: George Nicol (1794).
12. Wright AE, Douglas SR. An experimental investigation of the role of the blood fluids in connection with phagocytosis. *Proc R Soc Lond* (1903) **72**:357–70. doi:10.1098/rspl.1903.0062
13. Shaw GB. *The Doctor's Dilemma (play)* (1926). London: Constable.
14. Gall E. The cytological identity and interrelation of mesenchymal cells of lymphoid tissue. *Ann N Y Acad Sci* (1958) **73**:120–30. doi:10.1111/j.1749-6632.1959.tb40796.x
15. Ehrlich P. Methodologische beiträge zur physiologie und pathologie der verschiedenen formen der leukocyten. *Z Klin Med* (1880) **1**:553–60.
16. Florey HW. *General Pathology*. London: Lloyd-Luke (Medical Books) (1970).
17. Clark ER, Clark EL. Relation of monocytes of the blood to the tissue macrophages. *Am J Anat* (1930) **46**:149–85. doi:10.1002/aja.1000460105
18. Aschoff L. Das reticulo-endotheliale system. *Ergeb Inn Med Kinderheilkd* (1924) **26**:1–118.
19. Wintrobe MW. *Blood, Pure and Eloquent*. New York: McGraw-Hill Book Company (1980).
20. Sródka A, Gryglewski RW, Szczepariski W. Browicz or Kupffer cells? *Pol J Pathol* (2006) **57**:183–5.
21. Rous P, Beard JW. Selection with the magnet and cultivation of "reticuloendothelial" cells. *Science* (1933) **77**:92. doi:10.1126/science.77.1986.92
22. Rous P, Beard JW. Selection with the magnet and cultivation of reticuloendothelial cells (Kupffer cells). *J Exp Med* (1934) **59**:577–91. doi:10.1084/jem.59.5.577
23. Lewis MR, Lewis WH. Monocytes, macrophages, epithelioid cells, and giant cells. In: Sabin FR, Mayer AW, editors. *American Association of Anatomists*. Cleveland, OH: Western Reserve University (1925). p. 391.
24. Odelberg W. Baruch benacerraf les prix nobel. *Les Prix Nobel. The Nobel Prizes 1980*. Stockholm: Almqvist & Wiksell (1981).
25. Amor S, Smith PA, Hart B, Baker D. Biozzi mice: of mice and human neurological diseases. *J Neuroimmunol* (2005) **165**:1–10. doi:10.1016/j.jneuroim.2005.04.010
26. Rezaie P, Male D. Mesoglia & microglia – a historical review of the concept of mononuclear phagocytes within the central nervous system. *J Hist Neurosci* (2002) **11**:325–74. doi:10.1076/jhin.11.4.325.8531
27. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol Rev* (2011) **91**:461–553. doi:10.1152/physrev.00011.2010
28. Robertson WF. The normal histology and pathology of neuroglia. *J Ment Sci* (1897) **43**:733–52.
29. Robertson WF. *A Text-Book of Pathology in Relation to Mental Diseases*. Edinburgh: William F Clay (1900).
30. del Rio-Hortega P. The microglia. *Lancet* (1939) **233**:1023–6. doi:10.1016/S0140-6736(00)60571-8
31. Tomes TW, De Morgan C. Observations on the structure and development of bone. *Philos Trans* (1853) **143**:109–39. doi:10.1098/rstl.1853.0004
32. Kölliker A. *Die Normale Resorption des Knochengewebes und Ihre Bedeutung für Die Entstehung der Typischen Knochenformen*. Leipzig: von F.C.W. Vogel (1873).
33. Hancox NM. The osteoclast. *Biol Rev Camb Philos Soc* (1949) **24**:448–71. doi:10.1111/j.1469-185X.1949.tb00583.x
34. Wegner G. Myeloplaxen und knochenresorption. *Arch Pathol Anat Physiol Klin Med* (1872) **56**:523–33.
35. Arey LB. The origin, growth and fate of osteoclasts and their relation to bone resorption. *Am J Anat* (1920) **26**:314–45. doi:10.1002/aja.1000260302
36. Lyon M, Mollison PL. John Freeman Loutit 19 February 1910-11 June 1992. *Biogr Mems Fell R Soc* (1994) **40**:237–52. doi:10.1098/rsbm.1994.0037
37. Lang FJ. The reaction of lung tissue to tuberculous infection in vitro – two plates. *J Infect Dis* (1925) **37**:430–42. doi:10.1093/infdis/37.5.431
38. Robertson OH. Phagocytosis of foreign material in the lung. *Physiol Rev* (1941) **21**:112–39.
39. Karrer HE. The ultrastructure of mouse lung: the alveolar macrophage. *J Biophys Biochem Cytol* (1958) **4**:693–700. doi:10.1083/jcb.4.6.693
40. Maximow A. Role of the nongranular blood leukocytes in the formation of the tubercle. *J Infect Dis* (1925) **37**:418–29. doi:10.1093/infdis/37.5.418
41. McMaster PD, Heidelberger M. Florence Rena Sabin. *Natl Acad Sci Biograph Memoirs* (1960) **34**:270–319.
42. Lurie MB. Heredity, constitution and tuberculosis, an experimental study. *Am Rev Tuberc Suppl* (1941) **44**:1–125.
43. Mackaness GB. The growth of tubercle bacilli in monocytes from normal and vaccinated rabbits. *Am Rev Tuberc* (1954) **69**:495–504.
44. Miki K, Mackaness GB. The passive transfer of acquired resistance to *Listeria monocytogenes*. *J Exp Med* (1964) **120**:93–103. doi:10.1084/jem.120.1.93
45. North RJ. George B. Mackaness, M.D., D.Phil., F.R.S. *Tuberculosis* (2007) **87**:391. doi:10.1016/j.tube.2007.04.001
46. Dannenberg AM. *Pathogenesis of Human Pulmonary Tuberculosis: Insights from the Rabbit Model*. Washington, DC: ASM Press (2006).
47. Manwani D, Bieker JJ. The erythroblastic island. *Curr Top Dev Biol* (2008) **82**:23–53. doi:10.1016/S0070-2153(07)00002-6
48. Humphrey JL. Marginal zone and marginal sinus macrophages in the mouse are distinct populations. *Adv Exp Med Biol* (1979) **114**:381–8.
49. Humphrey JH. Splenic macrophages: antigen presenting cells for T1-2 antigens. *Immunol Lett* (1985) **11**:149–52. doi:10.1016/0165-2478(85)90161-0
50. Flemming W. Studien über regeneration der gewebe. *Arch mikroskopische Anat* (1885) **24**:50–91. doi:10.1007/BF02960374

51. Swartzendruber DC, Congdon CC. Electron microscope observations on tingible body macrophages in mouse spleen. *J Cell Biol* (1963) **19**:641–6. doi:10.1083/jcb.19.3.641
52. Cohn ZA. Determinants of infection in the peritoneal cavity. II. Factors influencing the fate of *Staphylococcus aureus* in the mouse. *Yale J Biol Med* (1962) **35**:29–47.
53. Awrorow PP, Timofejewskij AD. Kultivierungsversuche von leukämischem blute. *Virchows Arch A Pathol Pathol Anat* (1914) **216**:184–214.
54. Lewis MR, Lewis WH. The transformation of white blood cells – into clasmatocytes (macrophages), epithelioid cells, and giant cells. *J Am Med Assoc* (1925) **84**:798–9. doi:10.1001/jama.1925.02660370008002
55. Carrel A, Ebeling AH. The fundamental properties of the fibroblast and the macrophage: II. The macrophage. *J Exp Med* (1926) **44**:285–305. doi:10.1084/jem.44.2.261
56. Sabin FR, Doan CA, Cunningham RS. Discrimination of two types of phagocytic cells in the connective tissues by the supravital technique. *Contrib Embryol* (1925) **16**:125–62.
57. Ebert RH, Florey HW. The extravascular development of the monocyte observed in vivo. *Br J Exp Pathol* (1939) **20**:342–56.
58. Volkman A, Gowans JL. The origin of macrophages from the bone marrow in the rat. *Br J Exp Pathol* (1965) **46**:62–70.
59. Takahashi K. Development and differentiation of macrophages and related cell: historical review and current concepts. *J Clin Exp Hematop* (2001) **41**:1–33. doi:10.3960/jslrt.41.1
60. Marchesi VT, Florey HW. Electron micrographic observations on the emigration of leucocytes. *Q J Exp Physiol Cogn Med Sci* (1960) **45**:343–8.
61. Mildner A, Yona S, Jung S. A close encounter of the third kind: monocyte-derived cells. *Adv Immunol* (2013) **120**:69–103. doi:10.1016/B978-0-12-417028-5.00003-X
62. Konstantinov IE, Mejevoi N, Anichkov NM. Nikolai N. Anichkov and his theory of atherosclerosis. *Tex Heart Inst J* (2006) **33**:417–23.
63. Anitschkow N. Über die histogenese der myokardveränderungen bei einigen intoxikationen. *Virchows Arch A Pathol Pathol Anat* (1913) **211**:193–237.
64. Anitschkow N. Über die veränderung der kaninchenaorta bei experimenteller cholesterinsteatose. *Beitr Pathol Anat* (1913) **56**:379–404.
65. Steinberg D. *The Cholesterol Wars: The Skeptics vs the Preponderance of Evidence*. London: Academic Press (2007).
66. Dock W. Research in arteriosclerosis; the first fifty years. *Ann Intern Med* (1958) **49**:699–705. doi:10.7326/0003-4819-49-3-699
67. Willerson JT, Majesky MW, Fuster V. Russell Ross, PhD – visionary basic scientist in cardiovascular medicine – in memoriam. *Circulation* (2001) **103**:478–9. doi:10.1161/01.CIR.103.4.478
68. Aterman K, Remmele W, Smith M. Karl Touton and his "xanthelasmatic giant cell." A selective review of multinucleated giant cells. *Am J Dermatopathol* (1988) **10**:257–69.
69. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. [Mononuclear phagocytic system: new classification of macrophages, monocytes and of their cell line]. *Bull World Health Organ* (1972) **47**:651–8.
70. Maximow A. Über die entwicklung der blut-und bindegewebszellen beim säugetier-embryo. *Folia Haematol* (1907) **4**:611–26.
71. Mosier DE, Coppleson LW. A three-cell interaction required for the induction of the primary immune response in vitro. *Proc Natl Acad Sci U S A* (1968) **61**:542–7. doi:10.1073/pnas.61.2.542
72. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* (1973) **137**:1142–62. doi:10.1084/jem.137.5.1142
73. Nussenzweig MC, Steinman RM. Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *J Exp Med* (1980) **151**:1196–212. doi:10.1084/jem.151.5.1196
74. van Furth R. Identification of mononuclear phagocytes: overview and definitions. In: Adams DO, Edelson PJ, Koren H, editors. *Methods for Studying Mononuclear Phagocytes*. London: Academic Press (1980). p. 243–52.
75. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) **330**:841–5. doi:10.1126/science.1194637
76. Yamada M, Naito M, Takahashi K. Kupffer cell proliferation and glucan-induced granuloma formation in mice depleted of blood monocytes by strontium-89. *J Leukoc Biol* (1990) **47**:195–205.
77. Naito M, Hasegawa G, Takahashi K. Development, differentiation, and maturation of Kupffer cells. *Microsc Res Tech* (1997) **39**:350–64. doi:10.1002/(SICI)1097-0029(19971115)39:4<350::AID-JEMT5>3.3.CO;2-V
78. Alliot F, Godin I, Pessac B. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res* (1999) **117**:145–52. doi:10.1016/S0165-3806(99)00113-3
79. Lichanska AM, Hume DA. Origins and functions of phagocytes in the embryo. *Exp Hematol* (2000) **28**:601–11. doi:10.1016/S0301-472X(00)00157-0
80. Bigley V, Haniffa M, Doulatov S, Wang XN, Dickinson R, Mcgovern N, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. *J Exp Med* (2011) **208**:227–34. doi:10.1084/jem.20101459
81. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**:86–90. doi:10.1126/science.1219179
82. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**:792–804. doi:10.1016/j.immuni.2013.04.004
83. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**:79–91. doi:10.1016/j.immuni.2012.12.001
84. Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. *Immunity* (2014) **41**:21–35. doi:10.1016/j.immuni.2014.06.013
85. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2015) **518**:547–51. doi:10.1038/nature13989
86. Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, et al. C-myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* (2015) **42**:665–78. doi:10.1016/j.immuni.2015.03.011
87. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**:571–8. doi:10.1038/nri3712
88. Mildner A, Chapnik E, Manor O, Yona S, Kim KW, Aychek T, et al. Mononuclear phagocyte miRNome analysis identifies miR-142 as critical regulator of murine dendritic cell homeostasis. *Blood* (2013) **121**:1016–27. doi:10.1182/blood-2012-07-445999
89. Becher B, Schlitzer A, Chen JM, Mair F, Sumatoh HR, Teng KWW, et al. High-dimensional analysis of the murine myeloid cell system. *Nat Immunol* (2014) **15**:1181–9. doi:10.1038/ni.3006
90. Gautier EL, Ivanov S, Williams JW, Huang SCC, Marcelin G, Fairfax K, et al. Gata6 regulates aspartoacylase expression in resident peritoneal macrophages and controls their survival. *J Exp Med* (2014) **211**:1525–31. doi:10.1084/jem.20140570
91. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**:1327–40. doi:10.1016/j.cell.2014.11.023
92. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
93. Okabe Y, Medzhitov R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell* (2014) **157**:832–44. doi:10.1016/j.cell.2014.04.016
94. Rosas M, Davies LC, Giles PJ, Liao CT, Kharfan B, Stone TC, et al. The transcription factor Gata6 links tissue macrophage phenotype and proliferative renewal. *Science* (2014) **344**:645–8. doi:10.1126/science.1251414

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OPINION
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What's in a name? Some early and current issues in dendritic cell nomenclature

David Vremec[1] *and Ken Shortman*[1,2,3]*

[1] The Walter and Eliza Hall Institute, Melbourne, VIC, Australia, [2] Department of Medical Biology, The University of Melbourne, Melbourne, VIC, Australia, [3] Burnet Institute, Melbourne, VIC, Australia

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The name dendritic cell (DC) was given by Steinman to describe the unusual cell type he saw in spleen cell suspensions. This morphological description is not sufficient to specify the cell of so much interest to immunologists; many cells can adopt a similar form. A useful functional definition evolved as Steinman and colleagues explored the immunological properties of this novel cell type (1). DCs were considered as antigen collecting and processing cells able to present antigen on MHC molecules and efficiently activate even primary T-cells. Nowadays, immunologists would likely add to this definition, a capacity to sense the context in which the antigen was collected, via receptors for pathogen or damaged cell-derived material. Why might we need to go beyond the name "dendritic cell" for cells with these well-understood functions? Some limitations of this single name arose early in DC research. This article surveys some problems of definition encountered in past work from our own laboratory. The problems we encountered arose from two sources, the first the discovery of different DC subsets and the need to determine whether these represented different maturation states or separate sub-lineages. The second was the difficulty in distinguishing these DC subsets from macrophages.

Our first hint that there could be distinct types of DCs came from our studies with Wu and Ardavin on thymic T and DC development (2). We were surprised to find that a high proportion of mouse thymic DCs stained with antibodies against characteristic T-cell markers, such as CD8α; it was a relief to find they did not stain with antibodies against CD3 or the T-cell receptor! Pickup of material from thymocytes was eliminated as an explanation. We then found a similar but less frequent DC subset staining for surface CD8α among the DCs in mouse spleen and these DCs were shown to express mRNA for CD8α (3). Others had already reported some staining of DCs with anti-CD8; our work emphasized that these $CD8^+$ DCs were a distinct population, CD8α expression being positively correlated with expression of DEC205 but inversely correlated with expression of other markers such as CD4, CD11b, and, as illustrated in **Figure 1**, SIRPα (4, 5).

Immunological interest in the $CD8^+$ and $CD8^-$ DC subsets increased when it became apparent from the work of many laboratories that these DCs differed in immunological functions. Differences were apparent in the expression of toll-like and other microbial pattern recognition receptors, in the cytokines produced on activation, in the fate of the T-cells they stimulated, in their capacity to phagocytize dead cells, and in the processing of antigens for MHC class I versus MHC class II presentation [reviewed in Ref. (7)]. The key findings from our laboratory were that the $CD8^+$ DCs, when appropriately stimulated, were the most potent producers of IL12p70 (8), and that the $CD8^+$ DCs have a strong bias to cross-presenting exogenous antigens, both soluble and particulate, for MHC class I presentation (9, 10).

An important issue became whether these functionally distinct DC types represented different lineages, or were simply different maturation states within one very plastic lineage. There was direct evidence, confirmed by us, that some $CD8^-$ DCs could on adoptive transfer, produce $CD8^+$ DCs. However, these $CD8^+$ DCs proved to be generated from a small number of early members of the

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Martin Guilliams, Ghent University – VIB, Belgium

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Elodie Segura, Institut Curie, France
Steffen Jung, Weizmann Institute of Science, Israel

***Correspondence:**
Ken Shortman
shortman@wehi.edu.au

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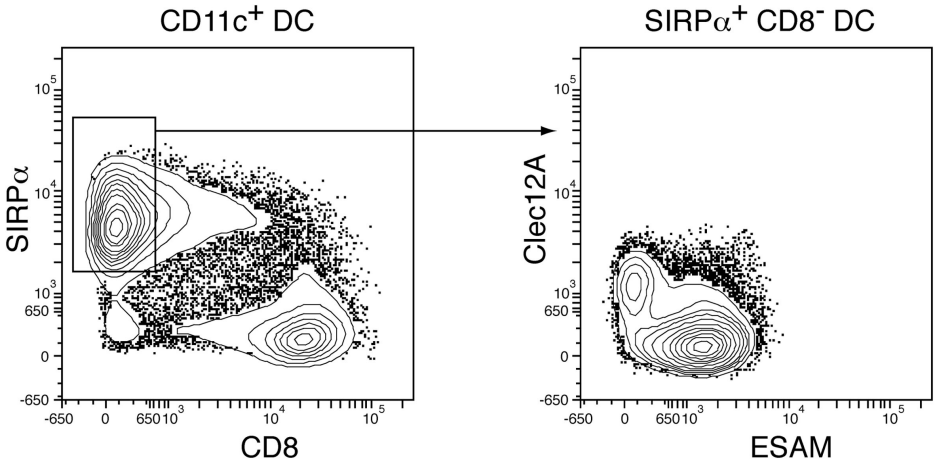

FIGURE 1 | Segregation of conventional DC subsets in mouse spleen. Spleen CD11c$^+$cDC were isolated, enriched, and gated as in Vremec et al. (5). Staining for CD8α and SIRPα allows clear segregation into the DC1 (CD8$^+$ DC) and DC2 (CD8$^-$ DC) subsets. However, the DC2 population can be separated into two further subsets by several surface markers, here Clec12A and ESAM as demonstrated in Lewis et al. (6).

CD8$^+$ DC lineage that had not yet acquired CD8α expression; the bulk of CD8$^-$ DCs did not give rise to CD8$^+$ DCs (11). Although sequential maturation states were found within the CD8$^+$ DC lineage, with early forms lacking CD8α expression (12) and the earliest stages lacking capacity for antigen cross-presentation (13), there was a clear developmental separation from the bulk of CD8$^-$ DCs. A further distinct DC type in mouse spleen became evident when the mouse equivalent of the human type 1 interferon-producing plasmacytoid dendritic cell (pDC) was identified (14). Although some pDCs expressed CD8α (15), they were clearly a separate lineage from the CD8$^+$ conventional DCs (cDCs). Our subsequent work with Naik showed that spleen CD8$^+$ DCs and CD8$^-$ DCs represented separate cDC sub-lineages derived via pre-DC populations from a bone marrow pro-DC or common dendritic cell precursor (CDP) (16–18). Thus, because of differences in surface phenotype, immunological functions, and developmental pathways, these two spleen cDC populations had to be distinguished, and the terms CD8$^+$ cDC and CD8$^-$ cDC became current.

In line with the pioneering work of Salomon et al. (19) and Anjuere et al. (20), we extended our analysis of DC subtypes from mouse spleen to mouse lymph nodes (LNs) (4, 21). Spleen should contain only what we termed the "lymphoid tissue resident" cDCs whereas LNs should contain both these and the "migratory" cDC type arriving via the lymph from other tissues. It was immediately apparent that the level of DC heterogeneity was greater than in spleen. One source of heterogeneity was the existence of different activation states within even one DC lineage. In particular, the DC that had migrated even in steady state from peripheral tissues such as skin into LNs were more activated than those remaining in skin, and more activated than their lymphoid tissue resident counterparts; the DCs that had migrated expressed higher surface levels of MHC class II and of co-stimulator molecules such as CD86. Although they were first called "mature" DCs they proved to be not fully activated but "semi-mature"; they were not producing cytokines and were likely tolerogenic (22). A similar transformation termed "spontaneous activation" occurred when spleen cDCs were isolated and placed in culture (23). In both cases, further signals, such as given by microbial products interacting with TLR ligands, were required to produce a fully active, cytokine secreting immunogenic DC. However, even when these different activation states were considered, further cDC subsets not found in spleen were apparent, such as the epidermal Langerhans cell-derived LN DCs. The full lineage complexity of LN has now been well delineated by other laboratories, a notable finding being the existence of a migratory form of the CD8$^+$ DC lineage but lacking CD8α expression, commonly termed as the CD103$^+$ cDCs (24–26).

Our second problem with DC nomenclature arose as we attempted to distinguish DCs from macrophages, a particularly difficult exercise in inflamed tissues. It was also difficult to relate the DC populations we isolated from steady state mouse spleen with the DCs produced by culture of monocytes with granulocyte-macrophage colony stimulating factor (GM-CSF), a well-established model of DC generation (27). At that time, it was generally assumed that all DCs and macrophages would be bone marrow derived cells, with monocytes as the common late precursor. Some questioned whether it was valid even to consider DCs as a separate entity rather than as a macrophage variant (28). We had some sympathy with this view, since in experiments with Metcalf we had difficulty in segregating DCs from macrophages in the peritoneal fluid from mice expressing high levels of GM-CSF (29). Although cells with DC function and surface phenotype could be segregated from macrophages at the extremes of the distribution, there appeared to be a continuum of phenotypes rather than two discrete populations. For us the clarification came when, with Naik, the immediate precursor of the spleen cDC was isolated and shown to be distinct from monocytes and unable to produce macrophages (16). We termed these pre-DCs. This led to the view that there were two different routes to cells with DC antigen presenting function, one via monocytes and more often found under conditions of inflammation, the other derived

from CDP/pro-DC precursors in bone marrow then via pre-DC to the types of DC found in steady state lymphoid tissue (17). The culture model finally developed for generation of the type of DCs found in steady state became bone marrow stimulated with Flt3 ligand, rather that with GM-CSF (30, 31). Thus, the developmental pathway leading to DC functions became a major criterion for segregating and naming DC subtypes.

It was then possible to segregate DCs derived from monocytes from the cDCs found in steady state spleen. However, it is evident from the account above that our previous nomenclature of the subsets of spleen cDCs based on CD8α expression was inadequate. Certain pDC subsets also expressed CD8α. Early DCs of "$CD8^+$ cDC" lineage in spleen did not express CD8α. The migratory version of the same lineage, the $CD103^+$ DCs, did not express it. And finally, CD8 was not expressed by human DCs. A major advance was the demonstration in several laboratories of an equivalent of the mouse "$CD8^+$ cDC" lineage within human DCs, and the finding that the chemokine receptor XCR1 and the C-type lectin-like molecule Clec9A, rather than CD8, served as common DC surface markers crossing this species barrier [commentary in Ref. (32)]. The proposed designation of this DC subtype as DC1 overcomes the previous nomenclature problems (33).

In contrast to these advances in understanding the DC1 subset, the $CD8^-$ $CD11b^{high}$ $SIRP\alpha^{high}$ cDCs (designated as DC2) have been less studied and still present nomenclature issues. We had already separated spleen $CD8^-$ DCs into two subsets based on CD4 expression (5), but the significance of this remains obscure. A more meaningful separation can now be made based on surface expression of Clec12A (DCAL2, MICL) versus DCIR2 or ESAM (6, 34, 35). An example of such segregation is shown in **Figure 1**. Importantly, these DC subsets differ in both developmental requirements and immunological characteristics; formation of $DCIR2^+$ $ESAM^{high}$ $Clec12A^-$ DCs requires Notch2 signaling and this subset selectively responds to flagellin and induces Th2 responses. Will these differences demand a further division into DC2 and DC3 subtypes? Or will one of these, particularly the $Clec12A^+$ subset, prove to be part of the monocyte-derived group? These questions require further work.

It is notable that ontogeny has led to a better understanding and provided one logical basis for DC classification (33). Will ontogeny be the best guide for DC nomenclature in future? We can foresee one area where it may cause confusion. A proportion of mouse pDCs and the CD8α-expressing subset of cDCs in the mouse thymus have a potential route of development from lymphoid rather than myeloid precursors (36, 37). These DC types have D–J rearrangements in their Ig heavy chain genes, a characteristic of lymphoid-origin cells (38). The extent to which a lymphoid route contributes to their development in steady state is still unclear, but the potential is there. Yet, the thymic $CD8^+$ DCs are similar to the splenic $CD8^+$ DCs of myeloid origin, and pDCs developing from myeloid or lymphoid precursors have similar surface phenotype and immunological functions. Should they have separate names according to their developmental origin, or should this "convergent" development lead to cells with the same name? There may yet be fine differences in function that eventually will be important to specify, but at present they are called by the same name. One resolution of this paradox comes from the likelihood that, despite the differences in bone marrow precursor surface markers, a common molecular program for pDC or for $CD8^+$ cDC formation has been initiated, with transcription factors that override any previous precursor orientation. Considering ontological origin in terms of these final molecular programs, rather than by the surface markers on the precursor cells, should overcome the paradox resulting from apparent convergent differentiation.

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References

1. Steinman RM. Decisions about dendritic cells: past, present and future. *Annu Rev Immunol* (2011) **30**:1–22. doi:10.1146/annurev-immunol-100311-102839
2. Shortman K, Wu L, Ardavin C, Vremec D, Stozik F, Winkel K, et al. Thymic dendritic cells: surface phenotype, developmental origin and function. In: Banchereau J, Schmitt D, editors. *Dendritic Cells in Fundamental and Clinical Immunology*. New York, NY: Plenum Publishing Corporation (1995). p. 21–9.
3. Vremec D, Zorbas M, Scollay R, Saunders DJ, Ardavin CF, Wu L, et al. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. *J Exp Med* (1992) **176**:47–58. doi:10.1084/jem.176.1.47
4. Vremec D, Shortman K. Dendritic cell subtypes in mouse lymphoid organs: cross-correlation of surface markers, changes with incubation, and differences among thymus, spleen, and lymph nodes. *J Immunol* (1997) **159**:565–73.
5. Vremec D, Pooley J, Hochrein H, Wu L, Shortman K. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. *J Immunol* (2000) **164**:2978–86. doi:10.4049/jimmunol.164.6.2978
6. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, et al. Notch2 receptor signalling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* (2011) **35**:780–91. doi:10.1016/j.immuni.2011.08.013
7. Shortman K, Heath WR. The $CD8^+$ dendritic cell subset. *Immunol Rev* (2010) **234**:18–31. doi:10.1111/j.0105-2896.2009.00870.x
8. Hochrein H, Shortman K, Vremec D, Scott B, Hertzog P, O'Keeffe M. Differential production of IL-12, IFN-alpha, and IFN-gamma by mouse dendritic cell subsets. *J Immunol* (2001) **166**:5448–55. doi:10.4049/jimmunol.166.9.5448
9. Pooley JL, Heath WR, Shortman K. Cutting edge: intravenous soluble antigen is presented to CD4 T cells by $CD8^-$ dendritic cells, but cross-presented to CD8 T cells by $CD8^+$ dendritic cells. *J Immunol* (2001) **166**:5327–30. doi:10.4049/jimmunol.166.9.5327
10. Schnorrer P, Behrens GM, Wilson NS, Pooley JL, Smith CM, El-Sukkari D, et al. The dominant role of $CD8^+$ dendritic cells in cross-presentation is not dictated by antigen capture. *Proc Natl Acad Sci U S A* (2006) **103**:10729–34. doi:10.1073/pnas.0601956103
11. Naik S, Vremec D, Wu L, O'Keeffe M, Shortman K. $CD8\alpha^+$ mouse spleen dendritic cells do not originate from the $CD8\alpha^-$ dendritic cell subset. *Blood* (2003) **102**:601–4. doi:10.1182/blood-2002-10-3186
12. Bedoui S, Prato S, Mintern J, Gebhardt T, Zhan Y, Lew A, et al. Characterisation of an intermediate splenic precursor of $CD8^+$ dendritic cells capable of inducing antiviral T cell responses. *J Immunol* (2009) **182**:4200–7. doi:10.4049/jimmunol.0802286
13. Sathe P, Pooley J, Vremec D, Mintern J, Jin JO, Wu L, et al. The acquisition of antigen cross-presentation function by newly formed dendritic cells. *J Immunol* (2011) **186**:5184–92. doi:10.4049/jimmunol.1002683

14. Asselin-Paturel C, Boonstra A, Dalod M, Durand I, Yessaad N, Dezutter-Dambuyant C, et al. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol* (2001) **2**:1144–50. doi:10.1038/ni736
15. O'Keeffe M, Hochrein H, Vremec D, Caminschi I, Miller JL, Anders EM, et al. Mouse plasmacytoid cells: long-lived cells, heterogeneous in surface phenotype and function, that differentiate into CD8α^+ dendritic cells only after microbial stimulus. *J Exp Med* (2002) **196**:1307–19. doi:10.1084/jem.20021031
16. Naik SH, Metcalf D, van Nieuwenhuijze A, Wicks I, Wu L, O'Keeffe M, et al. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat Immunol* (2006) **7**:663–71. doi:10.1038/ni1340
17. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* (2007) **7**:19–30. doi:10.1038/nri1996
18. Naik SH, Sathe P, Park H-Y, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single in vitro and in vivo-derived precursors. *Nat Immunol* (2007) **8**:1217–26. doi:10.1038/ni1522
19. Salomon B, Cohen JL, Masurier C, Klatzmann D. Three populations of mouse lymph node dendritic cells with different origins and dynamics. *J Immunol* (1998) **160**:708–17.
20. Anjuere F, Martin P, Ferraro I, Frage GM, del Hoyo GM, Wright N, et al. Definition of dendritic cell populations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse. *Blood* (1999) **93**:590–8.
21. Henri S, Vremec D, Kamath A, Waithman J, Williams S, Benoist C, et al. The dendritic cell populations of mouse lymph nodes. *J Immunol* (2001) **167**:741–8. doi:10.4049/jimmunol.167.2.741
22. Wilson NS, El-Sukkari D, Belz GT, Smith CM, Steptoe RJ, Heath WR, et al. Most lymphoid organ dendritic cell types are phenotypically and functionally immature. *Blood* (2003) **102**:2187–94. doi:10.1182/blood-2003-02-0513
23. Vremec D, O'Keeffe M, Wilson A, Ferrero I, Koch U, Radtke F, et al. Factors determining the spontaneous activation of splenic dendritic cells in culture. *Innate Immun* (2011) **17**:338–52. doi:10.1177/1753425910371396
24. Poulin LF, Henri S, de Bovis B, Devilard E, Kissenpfennig A, Malissen B. The dermis contains langerin+ dendritic cells that develop and function independently of epidermal Langerhans cells. *J Exp Med* (2007) **24**:3119–31. doi:10.1084/jem.20071724
25. Ginhoux F, Collin MP, Bogunovic M, Abel M, Leboeuf M, Helft J, et al. Blood-derived dermal langerin+ dendritic cells survey the skin in the steady state. *J Exp Med* (2007) **204**:3133–46. doi:10.1084/jem.20071733
26. Bursch LS, Wang L, Igyarto B, Kissenpfennig A, Malissen B, Kaplan DH, et al. Identification of a novel population of langerin$^+$ dendritic cells. *J Exp Med* (2007) **204**:3147–56. doi:10.1084/jem.20071966
27. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* (1994) **179**:1109–18. doi:10.1084/jem.179.4.1109
28. Hume DA. Macrophages as APC and the dendritic cell myth. *J Immunol* (2008) **181**:5829–35. doi:10.4049/jimmunol.181.9.5829
29. Metcalf D, Shortman K, Vremec D, Mifsud S, Di Rago L. Effects of excess GM-CSF levels on hematopoiesis and leukemia development in GM-CSF/max 41 double transgenic mice. *Leukemia* (1996) **10**:713–9.
30. Brasel K, De Smedt T, Smith JL, Maliszewski CR. Generation of murine dendritic cells from flt-3-ligand-supplemented bone marrow cultures. *Blood* (2000) **96**:3029–39.
31. Naik SH, Proietto AI, Wilson NS, Dakic A, Schnorrer P, Fuchsberger M, et al. Cutting edge: generation of splenic CD8$^+$ and CD8$^-$ dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. *J Immunol* (2005) **174**:6592–7. doi:10.4049/jimmunol.174.11.6592
32. Villadangos JA, Shortman K. Found in translation: the human equivalent of mouse CD8$^+$ dendritic cells. *J Exp Med* (2010) **207**:1131–4. doi:10.1084/jem.20100985
33. Guilliams M, Ginhoux F, Jakubzinck C, Naik S, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages – a proposal for a unifying nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**:571–8. doi:10.1038/nri3712
34. Lahoud MH, Proietto A, Ahmet F, Kitsoulis S, Eidsmo L, Wu L, et al. The C-type lectin Clec12A present on mouse and human dendritic cells can serve as a target for antigen delivery and enhancement of antibody responses. *J Immunol* (2009) **182**:7587–94. doi:10.4049/jimmunol.0900464
35. Kasahara S, Clark EA. Dendritic cell associated lectin 2 (DCAL2) defines a distinct CD8α dendritic cell subset. *J Leukoc Biol* (2012) **91**:418–37. doi:10.1189/jlb.0711384
36. Shortman K, Sathe P, Vremec D, Naik S, O'Keeffe M. Plasmacytoid dendritic cell development. *Adv Immunol* (2013) **120**:105–26. doi:10.1016/B978-0-12-417028-5.00004-1
37. Ardavin C, Wu L, Li CL, Shortman K. Thymic dendritic cells and T cells develop simultaneously within the thymus from a common precursor population. *Nature* (1993) **362**:761–3. doi:10.1038/362761a0
38. Corcoran L, Ferrero I, Vremec D, Lucas K, Waithman J, O'Keeffe M, et al. The lymphoid past of mouse plasmacytoid cells and thymic dendritic cells. *J Immunol* (2003) **170**:4926–32. doi:10.4049/jimmunol.170.10.4926

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A hitchhiker's guide to myeloid cell subsets: practical implementation of a novel mononuclear phagocyte classification system

Martin Guilliams[1,2]* *and Lianne van de Laar*[1,2]*

[1] *Laboratory of Immunoregulation, VIB Inflammation Research Center, Ghent University, Ghent, Belgium,* [2] *Department of Respiratory Medicine, University Hospital Ghent, Ghent, Belgium*

The classification of mononuclear phagocytes as either dendritic cells or macrophages has been mainly based on morphology, the expression of surface markers, and assumed functional specialization. We have recently proposed a novel classification system of mononuclear phagocytes based on their ontogeny. Here, we discuss the practical application of such a classification system through a number of prototypical examples we have encountered while hitchhiking from one subset to another, across species and between steady-state and inflammatory settings. Finally, we discuss the advantages and drawbacks of such a classification system and propose a number of improvements to move from theoretical concepts to concrete guidelines.

Keywords: nomenclature, dendritic cells, macrophages, monocytes, classification

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Edited by:
Ken J. Ishii,
National Institute of Biomedical Innovation, Japan

Reviewed by:
Richard A. Kroczek,
Robert Koch-Institute, Germany
Kazuhiro Suzuki,
Osaka University, Japan

***Correspondence:**
Martin Guilliams and Lianne van de Laar, Laboratory of Immunoregulation, VIB Inflammation Research Center, Ghent University, Technologiepark 927, Zwijnaarde, 9052 Ghent, Belgium
martin.guilliams@irc.vib-ugent.be; lianne.vandelaar@irc.vib-ugent.be

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Introduction

In the science fiction series created by Douglas Adams (1), the Hitchhiker's Guide to the Galaxy starts as follows: "*Space is big. Really big. You just won't believe how vastly hugely mind-boggling big it is. You may think it's a long way down the road to the chemist's, but that's just peanuts to space.*" Given the complexity of the mononuclear phagocyte (Star)system (MPS), one could easily give a similar warning to readers who are trying to make some sense of the huge number of hypothetically distinct dendritic cell (DC) and macrophage (MΦ) subsets. At the last International DC Symposium (DC2014, Tours – France), we counted at least 28 different DC subsets that were described using various surface markers and nomenclature systems in distinct species. If one would add the different MΦ subsets and the Cytof technology allowing to measure the expression of more than 30 different surface markers per cell, one could with a bit of luck end up with "42" as answer to the ultimate myeloid question of how many mononuclear phagocyte subsets exist in life, the universe, and everything. Although this would be great for fans of Douglas Adams, without Babel Fish to help us make some sense of so many different subsets, this evolution will not be beneficial for communication among myeloid cell experts, let alone for the communication toward pharmaceutical companies, scientific editors, medical doctors, or graduate students. We will here try to simplify this apparent complexity through a number of practical examples and theoretical concepts. Having hitchhiked from MΦ to DC labs studying myeloid cells in various tissues and in distinct inflammatory conditions, we would, in accordance with the Hitchhiker's Guide to the Galaxy, advise the following: do not panic and bring your towel along.

Members of the Mononuclear Phagocyte System

In the original MPS model proposed by Ralph van Furth, James Hirsch, and Zanvil Cohn, MΦs were proposed to derive from circulating monocytes (2). A couple of years later, Ralph Steinman and Zanvil Cohn identified DCs, which were also included in the MPS (3). The fact that DCs could be derived from human and mouse monocytes in GM-CSF-driven *in vitro* cultures (4–8) and *in vivo* upon inflammation or in barrier tissues (9–15) supported this concept. For a historical overview of the MPS field, we redirect the readers to the review of Simon Yona and Siamon Gordon in this issue (16). The identification of mouse hematopoietic precursors committed to the DC lineage called the common DC progenitors (CDPs – giving rise to pDCs and cDCs) and pre-cDCs (giving rise to cDCs) that are distinct from monocytes and can give rise to the so-called conventional DCs (cDCs) induced a first conceptual revolution in the field (12, 17–20). Moreover, Flt3-L, and not GM-CSF, was shown to be critically involved in the development of cDCs *in vitro* (8, 21–23) and *in vivo* (24–28). Recently, two additional committed precursors were identified in mice: the pre-pDC precursor that preferentially differentiates into pDCs (29), and the monocyte-committed common monocyte progenitor (cMop) (30). Importantly, the human equivalent of the pre-cDC, CDP, and cMop was recently identified (31, 32). A second conceptual revolution in the field was driven by the finding that most tissue-resident MΦs do not derive from circulating HSC-derived monocytes but develop from embryonic precursors, i.e., the yolk-sac MΦs (YS MΦs) or fetal liver (FL) monocytes (33–39). The relative contribution of YS MΦ-derived and FL monocyte-derived MΦs seems to vary from one organ to another (40–42). It was recently demonstrated that almost all MΦs have a YS origin [either directly from YS MΦs or through YS-derived EMPs (39)]. This may seem in contradiction with the proposed partial origin from FL monocytes (35, 43). However, it is now clear that YS-derived EMPs seed the FL and go through a FL monocyte intermediate before differentiating into most tissue-resident MΦs (44), reconciling most of the apparent discrepancies in the field. Together, these findings have challenged the MPS dogma and revealed that most DCs and MΦs derive from distinct committed precursors rather than from circulating HSC-derived monocytes (**Figure 1**).

Revisiting the Classification of Mononuclear Phagocytes

Historically, mononuclear phagocytes were classified as DCs or MΦs based on a restricted set of surface markers (CD11c and MHCII for DCs versus F4/80 for MΦs), proposed functional specialization (antigen-presentation and migration to lymph nodes for DCs versus phagocytosis for MΦs) and/or morphological features (dendritic-shaped cells for DCs versus large vacuolar cells for MΦs). However, these features are often not mutually exclusive. For example, although CD11c and MHCII are typically associated with DCs, alveolar MΦ are CD11c^{hi} and MHCII is expressed by intestinal MΦs (35, 45). Ideal surface markers allowing identification of the distinct myeloid cell subsets across tissues and species are still incomplete. Markers typically associated with some myeloid cell subsets can be lost or acquired by other subsets. The monocyte-associated

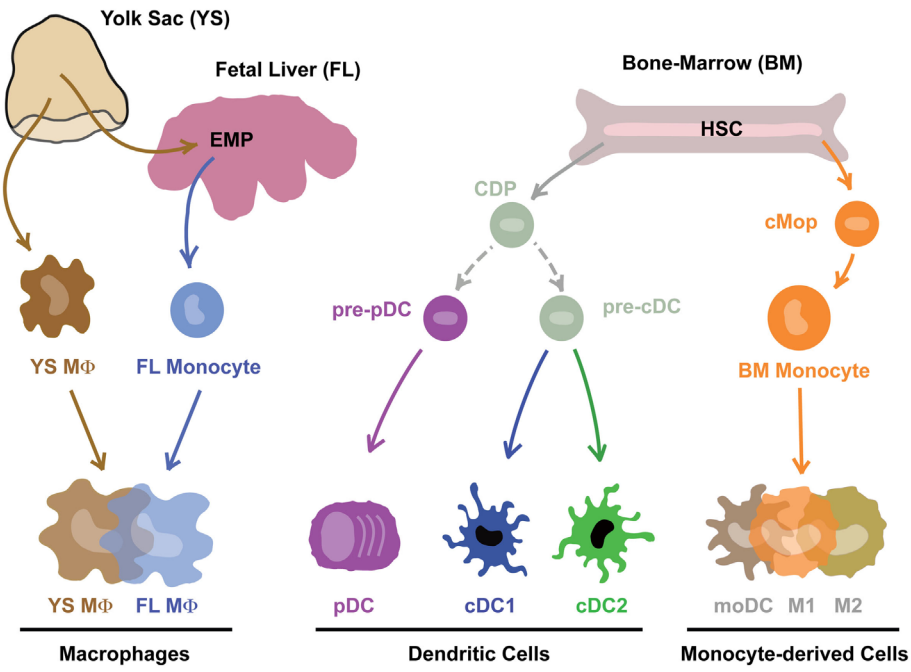

FIGURE 1 | Mononuclear phagocytes and their precursors. Note that this is work in progress and technical advances such as single-cell RNASeq and barcoding will in the near future prove or disprove many aspect of this theoretical scheme.

marker Ly-6C is rapidly down-regulated on many monocyte-derived cells (MCs) upon entrance in the tissues (45–48) and is expressed on pDCs (and lowly expressed on some cDCs). The pDC-associated marker mPDCA1 (stained with 120G8) can be acquired by MCs during inflammation (49). Alveolar MΦs (50) and Kupffer Cells (unpublished data) can upregulate CD11b during inflammation. Finally, BDCA3 is expressed on both human cDC1s and MCs (51). Thus, the inability to consistently identify myeloid cell subsets irrespective of tissue, species, or inflammatory state makes surface markers unattractive as basis for classification.

We would also propose to avoid a classification based primarily on functional specialization. First, each myeloid cell subset can perform more than one prototypical function. MΦs are often linked to phagocytosis of dead cells and pathogens but also have important immunomodulatory and metabolic functions. Second, subsets can acquire or lose functional capacities during inflammation as recently demonstrated for cDC2s that acquire cross-presentation capacities upon TLR stimulation (52). Therefore, we propose to disregard function as a basis for classifying cells.

Instead of surface markers, functional specialization, or morphology, we have recently suggested to classify cells based on their cellular origin, which could allow a more robust classification system (53). This would yield three big groups of cells (**Figure 1**): (i) embryonic progenitor-derived MΦs, (ii) CDP-derived DCs (that would be subdivided into cDC1s, cDC2s, and pDCs), and (iii) MCs. As these precursors have now been identified in both the mouse and the human, this allows one classification system across tissues and species.

Although precursor-based classification would provide a robust and species-conserved system, at the end of the day the function of the cells is what really matters for converting our knowledge into therapeutic advances for patients. Regrouping all the DCs into three big subsets of cDC1s, cDC2s, and pDCs will thus have the disadvantage of lumping together cells that may be in very different functional activation states. Similarly, MCs have been shown to be particularly plastic cells (54). Therefore, we propose to add a second classification level to the fixed ontogeny-based Level1 (**Figure 2**). Addition of a Level2 allows specification of the cellular activation state, the micro-anatomical localization or simply the surface markers utilized to identify the cells in a particular study. Of note, when defining the Level2 it will be important to avoid generalizations as a given function is often performed by only a fraction of the cells studied. We would thus propose to restrict the Level2 to objective criteria that can be measured at the single-cell level.

Practical Implementation for DCs

Historically, DCs were divided into subsets based on surface markers that differed between tissues and species, such as CD207 (Langerin) in the skin, CD103 (IntegrinαE) in the intestine, CD11b (IntegrinαM) in the lungs, CD4/CD8α in the spleen, and CD24/CD172α for *in vitro* differentiated DCs (**Figure 3**). Human DCs, on the other hand, have been divided into $CD141^+$ (BDCA3) and $CD1c^+$ (BDCA1) DCs. pDCs are identified by the expression of BDCA4 and BDCA2 in human, but by B220, mPDCA1 (BST2, recognized by 120G8), or Siglec-H in mice (53). Technical advances in multi-color flow cytometry have made matters worse with evermore "novel DC subsets" based on the expression of additional surface markers. By comparing the gene-expression profile of DCs isolated from various tissues and species, one can appreciate three big clusters of DCs (55–61). The pDC cluster includes mouse $PDCA1^+$ pDCs and human $BDCA2^+BDCA4^+$ pDCs. The cDC1 cluster comprises dermal $CD207^+CD103^+$ cDC1s, lung $CD103^+CD11b^-$cDC1s, splenic $CD8a^+CD4^-$ cDC1s, intestinal $CD103^+CD11b^-$ cDC1s and human blood $BDCA3^+$ cDC1s. Dermal $CD207^-CD11b^+$ cDC2s, lung $CD103^-CD11b^+$ cDC2s, splenic $CD8a^-CD4^+$ cDC2s, intestinal $CD103^+CD11b^+$ cDC2s and human blood $BDCA1^+$ cDC2s form the cDC2 cluster (62, 63). This bio-IT-driven analysis also revealed that within the cDC population, XCR1 and Sirpα are, respectively, expressed by all cDC1s and cDC2s across tissues, allowing an improved identification of these cells (51, 64–70). Note, however, that Sirpα is also expressed by other myeloid cells than cDC2s, showing the need for correct cDC identification prior to using this marker to distinguish cDC2s from cDC1s. Strikingly, this gene-expression-based division is supported by the existence of distinct pre-committed precursors (29, 71, 72) and by differential developmental transcription factor requirement of cDC1s,

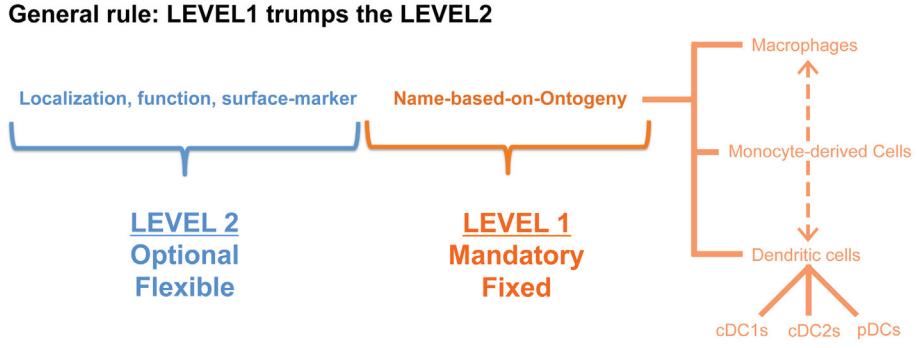

FIGURE 2 | A nomenclature system in two levels would have the advantage that cells can be first classified based on a restricted set of names (in this proposition according to their cellular origin: MΦ, MC, cDC1, cDC2, pDC) that would be applicable across species and across tissues, but the second level would still allow some flexibility to denote a distinct activation state or localization.

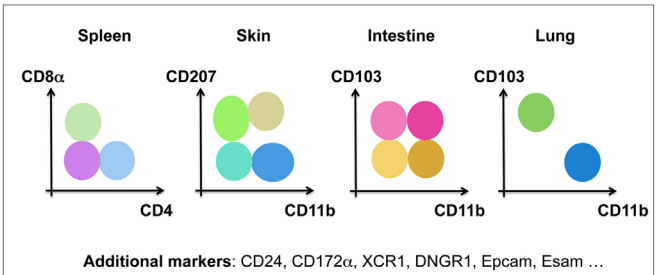

FIGURE 3 | Murine DCs have been subdivided into many different subsets based on distinct surface markers in the spleen, the skin, the intestine, and the lung.

cDC2s, and pDCs in the mouse. cDC1s, but not cDC2s, require BATF3 (71, 73, 74), ID2 (28, 75, 76), NFIL3 (77), and IRF8 (28, 71, 78–80) for their development, while cDC2s, but not cDC1s, are dependent on RELB (81), RBPJ (82), and IRF4 (79, 83–85). pDC development has been shown to be driven by E2-2 (86, 87).

The subdivision of DCs in three distinct Level1 groups is thus supported by their gene-expression profiles, cellular origin, and transcription factor requirement. However, these cells can acquire a distinct functional activation state from one tissue to another and in distinct inflammatory settings, underlining the need for a Level2 system. This can be illustrated by the capacity of intestinal cDCs to produce retinoic acid and promote the generation of induced regulatory T cells (iT_{REGs}) (88–91). Identification of DCs with superior iT_{REG} inducing ability is clinically relevant as the prevalence of food allergies, celiac disease and inflammatory bowel diseases is currently rising throughout the western world. Originally, it was described that $CD103^+$ but not $CD103^-$ intestinal DCs excel in iT_{REG} generation in a retinoic acid-dependent manner (89, 90). It is now clear that $CD103^+$ intestinal DCs comprise two ontogenically distinct subsets, $CD103^+CD11b^-$ cDC1s and $CD103^+CD11b^+$ cDC2s (74). Interestingly, rather than being associated with either of the two subsets, about half of the intestinal $CD103^+CD11b^-$ cDC1s were shown to possess the capacity to produce retinoic acid, while only one-third of the cDC2s do (91). Moreover, on a per cell basis retinoic acid producing $CD103^+$ cDC1s were the best at inducing iT_{REGs} (**Figure 4**). These data reveal that $CD103^+$ cDC1s, although broadly considered as a homogeneous subset, consist of 50% cells that are very efficient at inducing iT_{REGs} and 50% cells that are not. Interestingly, dermal cDC2s have higher retinoic acid-dependent iT_{REG} induction activity than dermal cDC1s (91). We hypothesize that this functional heterogeneity may be explained by the existence of distinct micro-environments within organs, inducing diverse functional modules on DCs. The finding that important functional modules can be acquired by only a fraction of cDC1s and/or cDC2s, which can moreover differ from one organ to another, illustrates the need for a Level2 nomenclature for DCs.

Another example of functional heterogeneity within DCs concerns the cDC2s. Splenic cDC2s contain a subpopulation that expresses CD4 and is specifically localized in the bridging channels (92). This localization has been shown to be EBI2-driven and essential to drive antibody production by B cells. The development of these $CD4^+$ cDC2s is Notch2 dependent. Note also that Notch2 deficiency is associated with defects in T_H17 induction (93, 94). In addition, it was found that KLF4 controls the development of a subpopulation of $CD24^{lo}CD11b^{lo}Sirp\alpha^{hi}$ cDC2s in the dermis (95). Importantly, loss of KLF4 was associated with loss of T_H2 induction. Thus, although cDC2s have been proposed to excel at both the induction of T_H2 (47, 96) and T_H17 responses (84, 85, 93), it may well be that these functional modules are in fact expressed by subpopulations of cDC2s (controlled by KLF4 and Notch2, respectively). In conclusion, although the current knowledge of early DC development in the bone-marrow seems to support only three big groups of DCs (cDC1s, cDC2s, and pDCs), it appears that a second layer of tissue-specific signals imprint operative gene modules on a fraction of DCs. Depending on their micro-localization, subpopulations of cDC1s, cDC2s, or pDCs will acquire distinct functional properties, requiring a flexible Level2 to classify and describe functionally distinct subpopulations.

A final example of a need for a Level2 classification involves inflammation-induced changes of surface marker expression. When mice are infected with the influenza virus, there is a transient change in the CD103 versus CD11b expression profile of lung cDCs, yielding four instead of two lung DC subsets (**Figure 5**). If one considers these as four distinct DC subsets, one could conclude that influenza infection disrupts hematopoiesis in the bone-marrow, as has been shown for Toxoplasma infection (97). Alternatively, these novel CD103/CD11b expression patterns may represent distinct local activation states of cDC1s or cDC2s. We have studied the cellular origin of the "novel" DC subsets arising during influenza infection (Neyt et al., manuscript in preparation). Our preliminary data suggest that $CD103^+CD11b^+$ cells are cDC2s that acquire CD103 expression during inflammation rather than a completely new subset. In conclusion, although we cannot rule out the existence of additional DC subsets that specifically develop during inflammation, when in doubt we propose to first evaluate whether cells with a novel surface receptor expression profile represent a different activation state of cDC1s or cDC2s before assuming the existence of a novel cDC3.

Practical Implementation for Embryonic Macrophages

In our classification system based on ontogeny all mononuclear phagocytes of embryonic origin are grouped together under a single Level1 as "macrophages" (**Figure 1**). This includes liver-resident Kupffer cells, brain-resident microglial cells, lung-resident alveolar MΦs but also epidermis-resident Langerhans cells. In effect, this would thus classify Langerhans cells as MΦs and not as DCs, based on the fact that these cells derive from embryonic precursors that seed the epidermis around birth and then self-maintain throughout life (43, 98). We propose to keep the historical names for MΦs with undisputed identities. Mouse Kupffer cells, for example, do not require a different nomenclature since these cells have a well-defined cellular origin [embryonic (34, 38, 39)], localization (i.e., the liver sinusoids), and gene-expression profile (99, 100). However, we would like to emphasize that not any $F4/80^+$ cell in the liver should be categorized as Kupffer cell. MCs infiltrating

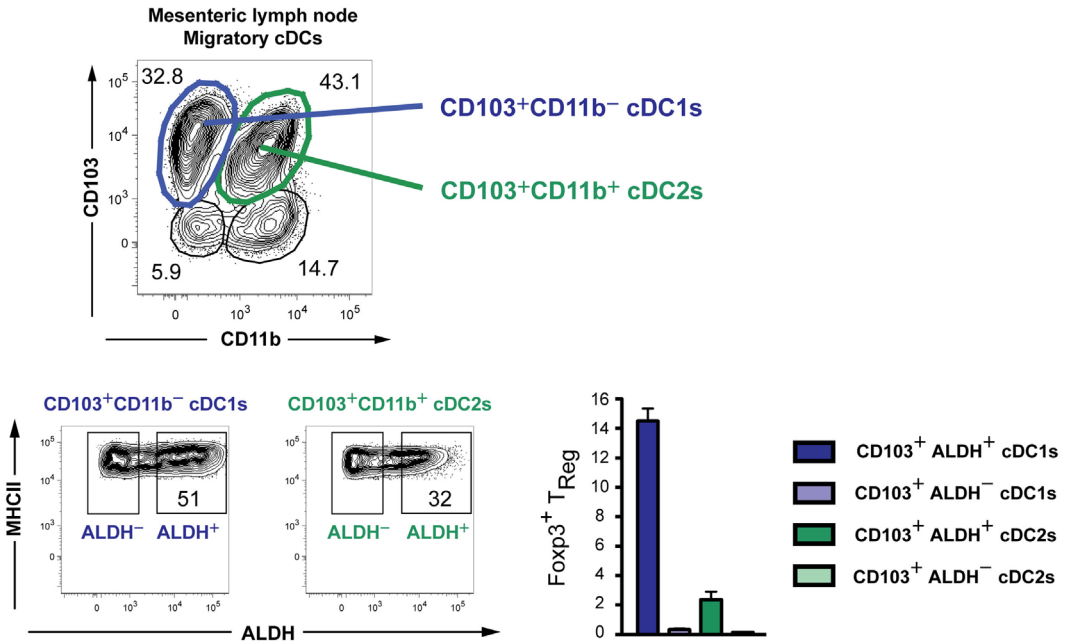

FIGURE 4 | Existence of subpopulations with distinct retinoic acid-producing capacities within both cDC1s and cDC2s in the mesenteric lymph nodes of mice. The capacity to produce retinoic acid was measured by the Aldefluor kit (91). DCs were sorted, loaded with the ovalbumin-peptide, and co-cultured *in vitro* with naïve OTII cells to measure the induction of Foxp3 on these cells.

the liver during acetaminophen-induced injury also express F4/80 but are short-lived and acquire a gene-expression profile that is strikingly different from resident Kupffer cells (100). Similarly, MCs infiltrating the central nervous system during inflammation are short-lived and do not acquire the specific gene-expression profile of embryonic microglia (101–103). As such, any MΦ-like cell in the liver or the brain should not be classified as Kupffer cell or microglia, respectively, as is often the case. Unfortunately, tools to correctly distinguish MCs from resident MΦs have long been lacking. In a way, this is surprising given the huge difference in gene-expression profile between resident embryonic MΦs and recruited MCs in these disease models. We have now identified several surface markers that are expressed by Kupffer cells but not MCs recruited during liver injury (Scott et al. manuscript in preparation) and we expect that given the striking heterogeneity of tissue-resident MΦs (104, 105) many of these MΦ-specific markers will be found. This will facilitate the correct classification of these cells and pave the way toward unraveling the functional differences between recruited MCs and tissue-resident embryonic MΦs during inflammation.

Practical Implementation for Monocyte-Derived Cells

Monocytes are particularly plastic cells. This can be appreciated using *in vitro* culture systems. Monocytes cultured with GM-CSF express some DC-like characteristics and have therefore long been referred to as moDCs. By contrast, culturing monocytes with M-CSF induces their differentiation into MΦ-like cells (moMΦs). Adding IL-4 or IFN-γ to M-CSF cultures further polarizes MCs into the so-called "classically activated MΦs/M1s" or "alternatively activated MΦs/M2s" (106), with strikingly different gene-expression profiles and metabolic modules (107). In a nomenclature system based on ontogeny, moDCs, M1s, or M2s are however first classified as MCs (Level1). In theory, this does not prevent further Level2 classification as "dendritic MCs," "classically activated MCs," or "alternatively activated MCs." However, we feel this polarized classification implies functional characteristics that are often not assessed experimentally. For example, MCs classified as M1s are typically associated with pathogen killing, M2s with wound healing, and moDCs with antigen-presentation (**Figure 6**). However, the identification of "dendritic MCs/moDCs," "classically activated MCs/M1s," or "alternatively activated MCs/M2s" *in vivo* turned out to be very challenging. In fact, profiling of MCs isolated from various inflamed tissues or *in vitro* culture systems reveals that monocytes can acquire a much broader transcriptional repertoire than suggested by the three-way M1/M2/moDC model. In recent efforts to further characterize the heterogeneity of MC activation states, Schultze and colleagues compared the gene-expression profile of MCs stimulated with a vast array of cytokines and TLR ligands. Instead of yielding a polarized model, the unbiased bio-informatics-driven clustering approach revealed a spectrum model (54). In our view, this spectrum model can be taken one step further and include the "dendritic MCs/moDCs" derived from GM-CSF-induced bone-marrow cultures as yet another extreme of the continuum of cellular faiths that can be acquired by monocytes. Rather

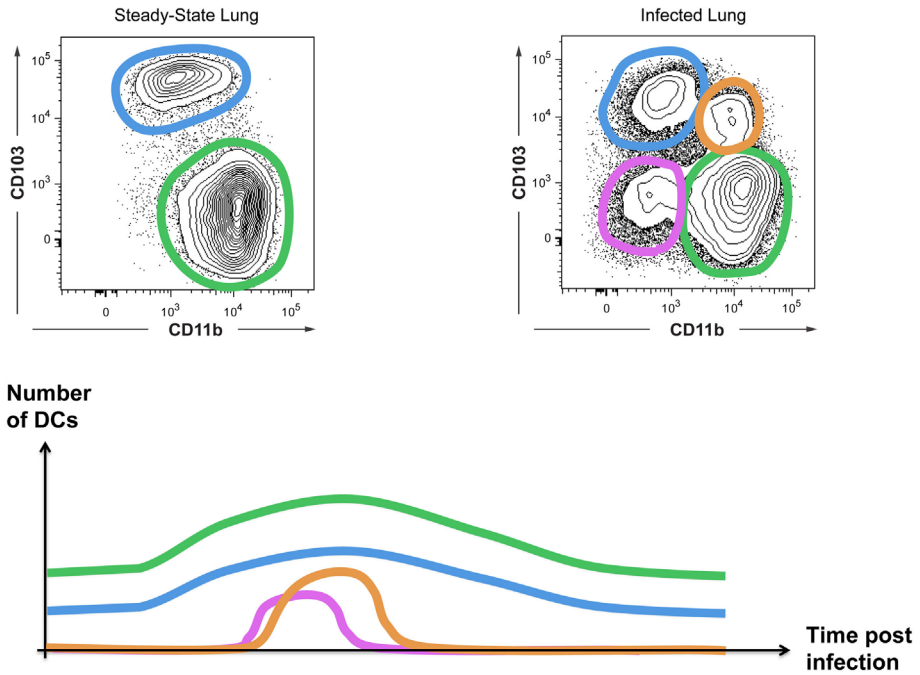

FIGURE 5 | Inflammation can induce the appearance of "novel" DC subsets. CD103 and CD11b expression on cDCs from uninfected or influenza-infected lungs are shown. The appearance of CD103^{+}CD11b^{+} DCs and CD103^{-}CD11b^{-} DCs is transient as schematically represented.

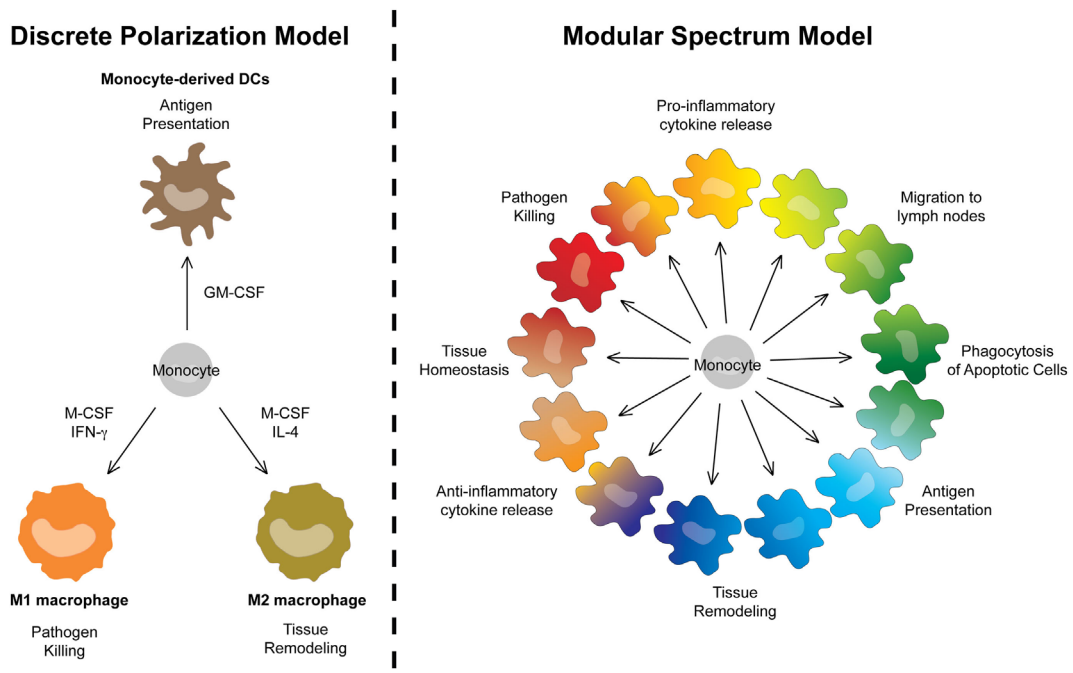

FIGURE 6 | A modular spectrum model for monocyte-derived cells. Replacement of the polarized three-way M1/M2/moDC model by a spectrum model in which bacterial killing, wound-healing, and antigen-presentation represent only three of many functional modules that can be acquired by MCs.

than unique end points, bacterial killing, wound-healing, and antigen-presentation represent three of many functional modules that can be acquired by MCs in a spectrum model that can be graphically represented by a continuous circle (**Figure 6**).

One important consequence of the herein-described classification would be the regrouping of moDCs and moMΦs under a single MC Level1. We feel this will represent an improvement for the field due to the lack of clear, mutually exclusive features

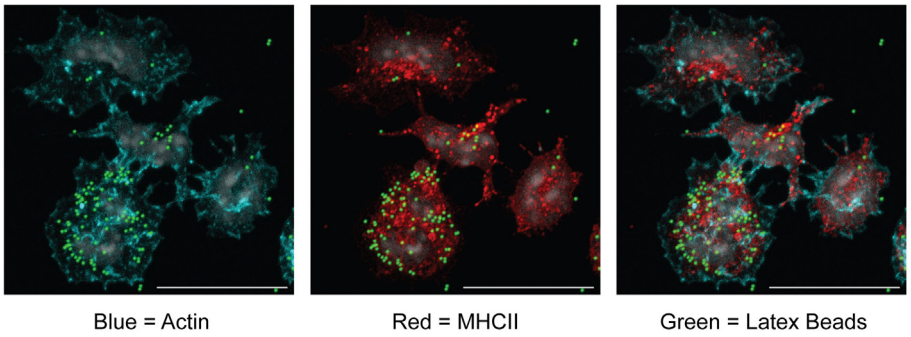

FIGURE 7 | Microscopic characterization of MCs isolated from inflamed mesenteric lymph nodes in an experimental model for colitis. CD11c^{hi}MHCII^{hi}CD64^{hi}F4/80^{hi} cells were sorted and cultured *in vitro* to evaluate their morphology and capacity to phagocytose latex beads (45).

that can be used to objectively separate moDCs from moMΦs (53). This problem can be illustrated by the MCs present within inflamed mesenteric lymph nodes during an experimental model for colitis (45). In this study, we called these cells moMΦs because they were CD64^{hi}F4/80^{hi} and excelled at phagocytosis (**Figure 7**). However, we could perfectly, like the Powrie group (108), have classified these cells as moDCs based on their CD11c^{hi}MHCII^{hi} profile, their localization within the T cell zone, their antigen-presentation capacity, or their dendritic morphology (**Figure 7**). By classifying these cells as CD64^{hi}F4/80^{hi} and/or CD11c^{hi}MHCII^{hi} MCs, they are recognized as one lineage, which will promote understanding and simplify communication between different research groups without preventing the study of DC-like or MΦ-like properties of specific MCs.

Finally, we do not expect MCs to be homogeneous in inflamed tissues. We and others found iNOS, the enzyme that is used by MΦs to produce NO and that is classically associated with an M1 activation state, to be typically expressed by only 10% of MCs (11, 45, 109). Since NO is bactericidal, suppressive for T cells, and can induce serious tissue damage, it makes perfect sense to study the factors that induce the expression of iNOS on a fraction of MCs. But by classifying these cells as "iNOS$^+$ MCs" instead of "M1 macrophages" or "TIP-DCs" one avoids to associate functions that have not been proven for these cells such as the antigen-presentation activity typically expected from DCs [in fact mice lacking monocytes showed identical T cell priming suggesting that TIP-DCs are not essential for this function (11)].

The Tough Cases Part I: moDCs as Fourth DC Subset?

MCs fitting the complete list of characteristics attributed to moDC, including migratory and antigen-presentation capacities comparable to cDCs, are not easily identified *in vivo*. We have described migratory MCs upon house-dust mite (HDM) exposure in the lungs (47), but their migration is much less efficient as compared to cDCs and required very high (and non-physiological) doses of HDM. In fact, we found that the majority of HDM-induced pulmonary MCs are not migratory cells but instead play an important role in the secretion of inflammatory chemokines that orchestrate the local immune responses. Similarly, a low-grade migration of CCR2$^+$CD64^{int} MCs was described upon DSS inflammation in the skin but this was minor as compared to cDC migration (48). Moreover, compared to cDCs these dermal CCR2$^+$CD64^{int} MCs displayed a rather modest antigen–antigen presentation capacity.

The most convincing pieces of evidence for cDC-like features of MCs come from *in vitro* culture systems. Bone-marrow cells cultured with GM-CSF yield cells with excellent antigen-presentation capacity that acquire CCR7, the chemokine receptor controlling migration of cDCs from tissues to lymph nodes, upon TLR stimulation and can migrate to the lymph nodes upon *in vivo* transfer (110, 111). This culture system has been used in many labs and is globally accepted to yield a homogeneous population of moDCs. This concept was first challenged by a study using single-cell transcriptomics (112). Among LPS-stimulated GM-CSF-induced bone-marrow-derived moDCs, the majority of cells were found to show high expression of inflammatory genes such as TNF, IL1, and CXCL10, while a smaller subset had much lower expression of these genes but displayed a signature reminiscent of "mature DCs," including high expression of CCR7 (113, 114). This was originally interpreted as functional heterogeneity among moDCs. However, in what we consider a landmark paper, Reis e Sousa and colleagues now demonstrate that this minor "mature" population in fact represents cDC2s that contaminate these cultures. These cDC2s displayed lower production of inflammatory cytokines but much better CCR7-ligand-induced migration and antigen-presentation as compared to GM-CSF-induced MCs (115, 116). This implies that many of the DC-like features of GM-CSF-induced moDCs should in fact not be attributed to MCs, but to a minor contaminating cDC2 population. All in all, both *in vitro* and *in vivo* data thus point toward a lower migration and antigen-presentation capacity of MCs as compared to cDCs, but conversely a higher production of inflammatory cytokines and chemokines. We therefore propose that in an inflamed organ the core business of cDCs will be migration to the draining lymph nodes and activation of naïve T cells, whereas MCs will primarily

orchestrate local inflammatory responses. Note that this has important consequences for DC-based vaccination strategies as this may explain why MC-based vaccines have only yielded modest clinical responses (117). The recent advances in cDC culture systems and the proper identification of committed circulating DC-precursors (31, 32, 118) may therefore pave the way toward more efficient cDC-based vaccination strategies.

The Tough Cases Part II: Steady-State MCs Versus Embryonic Macrophages

Most MΦ-like cells present in steady-state tissues are of embryonic origin (33–36, 38, 39, 44). However, puzzling exceptions have been reported. Although embryonic MΦs colonize the intestine and the heart before birth, these cells are thereafter progressively replaced by MCs. Importantly, these cells are relatively short-lived and continuous monocyte-recruitment is required to maintain the MC pool in these tissues (45, 46, 119–121). Similarly, monocytes are continuously recruited to the steady-state dermis (48). Therefore, while in some steady-state tissues, including the lung and the spleen, monocytes have been proposed to remain in an undifferentiated state (37, 122); in others, including the intestine, the skin, and the heart, they acquire a MΦ-like phenotype. The classification of MCs that differentiate in these steady-state organs and that replace the embryonic MΦs is difficult. They do not fit the profile of the MCs that are recruited to inflamed tissues, including pulmonary infection (36), auto-immune brain inflammation (101, 102), and acute liver injury (100), since in these inflammatory settings MCs do not replace the embryonic MΦs and display a very different gene-expression profile. Future research will be required to compare the functional properties and gene-expression profile of the embryonic MΦs present in the intestine, the skin, and the heart to the ones from the MCs that replace them with time. It will be interesting to compare the influence of tissue-imprinting to the intrinsic differences associated with their distinct cellular origin. Embryonic MΦs were recently compared to their bone-marrow-derived counterparts that replace them after irradiation-induced depletion. It was found that both cells share between 50 and 90% of the tissue-specific epigenetic landscape (105). This emphasizes the importance of tissue-imprinting, but at the same time implies that between 10 and 50% of the epigenetic landscape could be governed by the cellular origin of the cells. Future research will be required to assess the functional relevance of these findings. In the meantime, the classification of MΦ-like MCs in steady-state tissues remains difficult.

The Way Forward

The Level1 that forms the scaffold of the herein proposed classification system is in part based on elegant murine fate-mapping systems developed to study the cellular origin of MΦs (33, 34, 38, 39, 43, 44) and DCs [(123) and (124) in this issue]. Although the recent identification of committed DC-precursors distinct from monocytes in humans suggests that many of the principles identified in mice apply to the human immune system, this remains to be formally proven. Moreover, many of these murine fate-mapping systems label only a small fraction of the cells per population, rendering functional studies difficult. In cases where classification as cDC1, cDC2, pDC, MC, or MΦ is not obvious, a core set of signature genes that are specific for each cell type could facilitate correct Level1 identification. However, such signature genes are not easily identified. In addition, identification based on surface receptors would be most practical since it would allow the sorting of living cells through flow cytometry for functional assays. Ideally, such markers would be conserved across species. We are currently data-mining the gene-expression profiles of cells from various tissues and species to try to identify such markers. This can however represent a catch22. To find markers specifically expressed by the different populations, one requires pure gene-expression profiles, but correct sorting of the cells without contamination by other populations for RNA profiling requires the very markers we are looking for. Recent technological advances in single-cell RNA sequencing will allow to profile the gene expression of mixed populations. This may at last disentangle mixed myeloid populations and will hopefully provide the field with new markers that can then be validated with the current fate-mapping systems.

Although the current classification systems should thus be seen as work in progress, we are confident that in the near future better markers will be found which faithfully translate the cellular origin of cells and will form a practical base for the Level1 classification of myeloid cells. The Level2 classification should in our view be kept as flexible as possible to allow researchers to focus on one particular functional attribute of their cells of interest without implying too many additional features that have not been studied. Finally, it is noteworthy that in parallel to our proposition a nomenclature system for MΦs was proposed (106). In this proposition, terms implying functional specialization such as "classically activated macrophages" (pro-inflammatory) or "alternatively activated macrophages" (anti-inflammatory) were replaced by an objective description of how a MΦ is cultured *in vitro* [e.g., MΦ (IL-10)] or identified *in vivo* (e.g., "Relma^{hi} MΦ"). Thus, the common core Level1 would be MΦ and the added description provides the Level2. This and our classification system thus share three important principles: (i) elimination of terms that imply functional specialization as much as possible, (ii) introduction of a fixed Level1 system across species and tissues, and (iii) permitting flexibility through a Level2 system. Irrespective of which system is used to define the Level1 [ontogeny as we propose, or gene-expression profile as proposed by the Dalod group in this issue (60, 61)], we feel these three principles should be maintained for a future and hopefully definitive classification system.

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References

1. Adams D. *The Hitch Hiker's Guide to the Galaxy*. London: Pan Books (1979).
2. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. [Mononuclear phagocytic system: new classification of macrophages, monocytes and of their cell line]. *Bull World Health Organ* (1972) **47**:651–8.
3. van Furth R. "Identification of Mononuclear Phagocytes: Overview and Definitions," in *Methods for Studying Mononuclear Phagocytes*. London: Academic Press (1980). p. 243–52.
4. Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, et al. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* (1992) **176**:1693–702. doi:10.1084/jem.176.6.1693
5. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* (1994) **179**:1109–18. doi:10.1084/jem.179.4.1109
6. Caux C, Vanbervliet B, Massacrier C, Dezutter-Dambuyant C, De Saint-Vis B, Jacquet C, et al. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. *J Exp Med* (1996) **184**:695–706. doi:10.1084/jem.184.2.695
7. Palucka KA, Taquet N, Sanchez-Chapuis F, Gluckman JC. Dendritic cells as the terminal stage of monocyte differentiation. *J Immunol* (1998) **160**:4587–95.
8. Xu Y, Zhan Y, Lew AM, Naik SH, Kershaw MH. Differential development of murine dendritic cells by GM-CSF versus Flt3 ligand has implications for inflammation and trafficking. *J Immunol* (2007) **179**:7577–84. doi:10.4049/jimmunol.179.11.7577
9. Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunity* (1999) **11**:753–61. doi:10.1016/S1074-7613(00)80149-1
10. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* (2003) **19**:71–82. doi:10.1016/S1074-7613(03)00174-2
11. Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* (2003) **19**:59–70. doi:10.1016/S1074-7613(03)00171-7
12. Naik SH, Metcalf D, Van Nieuwenhuijze A, Wicks I, Wu L, O'Keeffe M, et al. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat Immunol* (2006) **7**:663–71. doi:10.1038/ni1340
13. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* (2007) **204**:171–80. doi:10.1084/jem.20061011
14. Bogunovic M, Ginhoux F, Helft J, Shang L, Hashimoto D, Greter M, et al. Origin of the lamina propria dendritic cell network. *Immunity* (2009) **31**(3):513–25. doi:10.1016/j.immuni.2009.08.010
15. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity* (2009) **31**:502–12. doi:10.1016/j.immuni.2009.06.025
16. Yona S, Gordon S. From the reticulo-endothelial to mononuclear phagocyte system – the unaccounted years. *Front Immunol* (2015) **6**:328. doi:10.3389/fimmu.2015.00328
17. Diao J, Winter E, Cantin C, Chen W, Xu L, Kelvin D, et al. In situ replication of immediate dendritic cell (DC) precursors contributes to conventional DC homeostasis in lymphoid tissue. *J Immunol* (2006) **176**:7196–206. doi:10.4049/jimmunol.176.12.7196
18. Naik SH, Sathe P, Park HY, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol* (2007) **8**:1217–26. doi:10.1038/ni1522
19. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, Manz MG. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol* (2007) **8**:1207–16. doi:10.1038/ni1518
20. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* (2013) **31**:563–604. doi:10.1146/annurev-immunol-020711-074950
21. Saunders D, Lucas K, Ismaili J, Wu L, Maraskovsky E, Dunn A, et al. Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony-stimulating factor. *J Exp Med* (1996) **184**:2185–96. doi:10.1084/jem.184.6.2185
22. Pulendran B, Banchereau J, Burkeholder S, Kraus E, Guinet E, Chalouni C, et al. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J Immunol* (2000) **165**:566–72. doi:10.4049/jimmunol.165.1.566
23. Naik SH, Proietto AI, Wilson NS, Dakic A, Schnorrer P, Fuchsberger M, et al. Cutting edge: generation of splenic CD8+ and CD8- dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. *J Immunol* (2005) **174**:6592–7. doi:10.4049/jimmunol.174.11.6592
24. Salomon B, Cohen JL, Masurier C, Klatzmann D. Three populations of mouse lymph node dendritic cells with different origins and dynamics. *J Immunol* (1998) **160**:708–17.
25. McKenna HJ, Stocking KL, Miller RE, Brasel K, De Smedt T, Maraskovsky E, et al. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* (2000) **95**:3489–97.
26. Karsunky H, Merad M, Cozzio A, Weissman IL, Manz MG. Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo. *J Exp Med* (2003) **198**:305–13. doi:10.1084/jem.20030323
27. Waskow C, Liu K, Darrasse-Jeze G, Guermonprez P, Ginhoux F, Merad M, et al. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol* (2008) **9**:676–83. doi:10.1038/ni.1615
28. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* (2009) **206**:3115–30. doi:10.1084/jem.20091756
29. Onai N, Kurabayashi K, Hosoi-Amaike M, Toyama-Sorimachi N, Matsushima K, Inaba K, et al. A clonogenic progenitor with prominent plasmacytoid dendritic cell developmental potential. *Immunity* (2013) **38**:943–57. doi:10.1016/j.immuni.2013.04.006
30. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* (2013) **14**:821–30. doi:10.1038/ni.2638
31. Breton G, Lee J, Zhou YJ, Schreiber JJ, Keler T, Puhr S, et al. Circulating precursors of human CD1c+ and CD141+ dendritic cells. *J Exp Med* (2015) **212**:401–13. doi:10.1084/jem.20141441
32. Lee J, Breton G, Oliveira TY, Zhou YJ, Aljoufi A, Puhr S, et al. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. *J Exp Med* (2015) **212**:385–99. doi:10.1084/jem.20141442
33. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) **330**:841–5. doi:10.1126/science.1194637
34. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**:86–90. doi:10.1126/science.1219179
35. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* (2013) **210**:1977–92. doi:10.1084/jem.20131199
36. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**:792–804. doi:10.1016/j.immuni.2013.04.004
37. Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, et al. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* (2013) **39**:599–610. doi:10.1016/j.immuni.2013.08.007
38. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**:79–91. doi:10.1016/j.immuni.2012.12.001
39. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2015) **518**:547–51. doi:10.1038/nature13989

40. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol* (2014) **14**:392–404. doi:10.1038/nri3671
41. Scott CL, Henri S, Guilliams M. Mononuclear phagocytes of the intestine, the skin, and the lung. *Immunol Rev* (2014) **262**:9–24. doi:10.1111/imr.12220
42. Varol C, Mildner A, Jung S. Macrophages: development and tissue specialization. *Annu Rev Immunol* (2015) **33**:643–75. doi:10.1146/annurev-immunol-032414-112220
43. Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med* (2012) **209**(6):1167–81. doi:10.1084/jem.20120340
44. Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, et al. C-myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* (2015) **42**:665–78. doi:10.1016/j.immuni.2015.03.011
45. Tamoutounour S, Henri S, Lelouard H, De Bovis B, De Haar C, Van Der Woude CJ, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol* (2012) **42**(12):3150–66. doi:10.1002/eji.201242847
46. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6C(hi) monocyte precursors. *Mucosal Immunol* (2012) **6**(3):498–510. doi:10.1038/mi.2012.89
47. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* (2013) **38**:322–35. doi:10.1016/j.immuni.2012.10.016
48. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* (2013) **39**:925–38. doi:10.1016/j.immuni.2013.10.004
49. Geurtsvankessel CH, Bergen IM, Muskens F, Boon L, Hoogsteden HC, Osterhaus AD, et al. Both conventional and interferon killer dendritic cells have antigen-presenting capacity during influenza virus infection. *PLoS One* (2009) **4**:e7187. doi:10.1371/journal.pone.0007187
50. Janssen WJ, Barthel L, Muldrow A, Oberley-Deegan RE, Kearns MT, Jakubzick C, et al. Fas determines differential fates of resident and recruited macrophages during resolution of acute lung injury. *Am J Respir Crit Care Med* (2011) **184**:547–60. doi:10.1164/rccm.201011-1891OC
51. Balan S, Ollion V, Colletti N, Chelbi R, Montanana-Sanchis F, Liu H, et al. Human XCR1+ dendritic cells derived in vitro from CD34+ progenitors closely resemble blood dendritic cells, including their adjuvant responsiveness, contrary to monocyte-derived dendritic cells. *J Immunol* (2014) **193**:1622–35. doi:10.4049/jimmunol.1401243
52. Desch AN, Gibbings SL, Clambey ET, Janssen WJ, Slansky JE, Kedl RM, et al. Dendritic cell subsets require cis-activation for cytotoxic CD8 T-cell induction. *Nat Commun* (2014) **5**:4674. doi:10.1038/ncomms5674
53. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**:571–8. doi:10.1038/nri3712
54. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* (2014) **40**:274–88. doi:10.1016/j.immuni.2014.01.006
55. Robbins SH, Walzer T, Dembele D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* (2008) **9**:R17. doi:10.1186/gb-2008-9-1-r17
56. Crozat K, Guiton R, Guilliams M, Henri S, Baranek T, Schwartz-Cornil I, et al. Comparative genomics as a tool to reveal functional equivalences between human and mouse dendritic cell subsets. *Immunol Rev* (2010) **234**:177–98. doi:10.1111/j.0105-2896.2009.00868.x
57. Guilliams M, Henri S, Tamoutounour S, Ardouin L, Schwartz-Cornil I, Dalod M, et al. From skin dendritic cells to a simplified classification of human and mouse dendritic cell subsets. *Eur J Immunol* (2010) **40**:2089–94. doi:10.1002/eji.201040498
58. Miller MA, Feng XJ, Li G, Rabitz HA. Identifying biological network structure, predicting network behavior, and classifying network state with high dimensional model representation (HDMR). *PLoS One* (2012) **7**:e37664. doi:10.1371/journal.pone.0037664
59. Vu Manh TP, Marty H, Sibille P, Le Vern Y, Kaspers B, Dalod M, et al. Existence of conventional dendritic cells in gallus gallus revealed by comparative gene expression profiling. *J Immunol* (2014) **192**(10):4510–7. doi:10.4049/jimmunol.1303405
60. Vu Manh TP, Bertho N, Hosmalin A, Schwartz-Cornil I, Dalod M. Investigating evolutionary conservation of dendritic cell subset identity and functions. *Front Immunol* (2015) **6**:260. doi:10.3389/fimmu.2015.00260
61. Vu Manh TP, Elhmouzi-Younes J, Urien C, Ruscanu S, Jouneau L, Bourge M, et al. Defining mononuclear phagocyte subset homology across several distant warm-blooded vertebrates through comparative transcriptomics. *Front Immunol* (2015) **6**:299. doi:10.3389/fimmu.2015.00299
62. Durand M, Segura E. The known unknowns of the human dendritic cell network. *Front Immunol* (2015) **6**:129. doi:10.3389/fimmu.2015.00129
63. Reynolds G, Haniffa M. Human and mouse mononuclear phagocyte networks: a tale of two species? *Front Immunol* (2015) **6**:330. doi:10.3389/fimmu.2015.00330
64. Bachem A, Guttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J Exp Med* (2010) **207**:1273–81. doi:10.1084/jem.20100348
65. Contreras V, Urien C, Guiton R, Alexandre Y, Vu Manh TP, Andrieu T, et al. Existence of CD8alpha-like dendritic cells with a conserved functional specialization and a common molecular signature in distant mammalian species. *J Immunol* (2010) **185**:3313–25. doi:10.4049/jimmunol.1000824
66. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, Ventre E, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**:1283–92. doi:10.1084/jem.20100223
67. Crozat K, Tamoutounour S, Vu Manh TP, Fossum E, Luche H, Ardouin L, et al. Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the CD8alpha+ type. *J Immunol* (2011) **187**:4411–5. doi:10.4049/jimmunol.1101717
68. Bachem A, Hartung E, Guttler S, Mora A, Zhou X, Hegemann A, et al. Expression of XCR1 characterizes the Batf3-dependent lineage of dendritic cells capable of antigen cross-presentation. *Front Immunol* (2012) **3**:214. doi:10.3389/fimmu.2012.00214
69. Becker M, Guttler S, Bachem A, Hartung E, Mora A, Jakel A, et al. Ontogenic, phenotypic, and functional characterization of XCR1(+) dendritic cells leads to a consistent classification of intestinal dendritic cells based on the expression of XCR1 and SIRPalpha. *Front Immunol* (2014) **5**:326. doi:10.3389/fimmu.2014.00326
70. Gurka S, Hartung E, Becker M, Kroczek RA. Mouse conventional dendritic cells can be universally classified based on the mutually exclusive expression of XCR1 and SIRPalpha. *Front Immunol* (2015) **6**:35. doi:10.3389/fimmu.2015.00035
71. Grajales-Reyes GE, Iwata A, Albring J, Wu X, Tussiwand R, Kc W, et al. Batf3 maintains autoactivation of Irf8 for commitment of a CD8alpha conventional DC clonogenic progenitor. *Nat Immunol* (2015) **16**(7):708–17. doi:10.1038/ni.3197
72. Schlitzer A, Sivakamasundari V, Chen J, Sumatoh HR, Schreuder J, Lum J, et al. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. *Nat Immunol* (2015) **16**(7):718–28. doi:10.1038/ni.3200
73. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* (2008) **322**:1097–100. doi:10.1126/science.1164206
74. Edelson BT, Kc W, Juang R, Kohyama M, Benoit LA, Klekotka PA, et al. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J Exp Med* (2010) **207**:823–36. doi:10.1084/jem.20091627

75. Hacker C, Kirsch RD, Ju XS, Hieronymus T, Gust TC, Kuhl C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat Immunol* (2003) **4**:380–6. doi:10.1038/ni903
76. Jackson JT, Hu Y, Liu R, Masson F, D'Amico A, Carotta S, et al. Id2 expression delineates differential checkpoints in the genetic program of CD8alpha+ and CD103+ dendritic cell lineages. *EMBO J* (2011) **30**:2690–704. doi:10.1038/emboj.2011.163
77. Kashiwada M, Pham NL, Pewe LL, Harty JT, Rothman PB. NFIL3/E4BP4 is a key transcription factor for CD8alpha(+) dendritic cell development. *Blood* (2011) **117**:6193–7. doi:10.1182/blood-2010-07-295873
78. Schiavoni G, Mattei F, Sestili P, Borghi P, Venditti M, Morse HC III, et al. ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells. *J Exp Med* (2002) **196**:1415–25. doi:10.1084/jem.20021263
79. Tamura T, Tailor P, Yamaoka K, Kong HJ, Tsujimura H, O'Shea JJ, et al. IFN regulatory factor-4 and -8 govern dendritic cell subset development and their functional diversity. *J Immunol* (2005) **174**:2573–81. doi:10.4049/jimmunol.174.5.2573
80. Tailor P, Tamura T, Morse HC III, Ozato K. The BXH2 mutation in IRF8 differentially impairs dendritic cell subset development in the mouse. *Blood* (2008) **111**:1942–5. doi:10.1182/blood-2007-07-100750
81. Wu L, D'Amico A, Winkel KD, Suter M, Lo D, Shortman K. RelB is essential for the development of myeloid-related CD8alpha- dendritic cells but not of lymphoid-related CD8alpha+ dendritic cells. *Immunity* (1998) **9**:839–47. doi:10.1016/S1074-7613(00)80649-4
82. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J Exp Med* (2007) **204**:1653–64. doi:10.1084/jem.20062648
83. Suzuki S, Honma K, Matsuyama T, Suzuki K, Toriyama K, Akitoyo I, et al. Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha- dendritic cell development. *Proc Natl Acad Sci U S A* (2004) **101**:8981–6. doi:10.1073/pnas.0402139101
84. Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hagerbrand K, Marsal J, et al. IRF4 transcription-factor-dependent CD103CD11b dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* (2013) **38**(5):958–69. doi:10.1016/j.immuni.2013.03.009
85. Schlitzer A, Mcgovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* (2013) **38**:970–83. doi:10.1016/j.immuni.2013.04.011
86. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* (2008) **135**:37–48. doi:10.1016/j.cell.2008.09.016
87. Ghosh HS, Cisse B, Bunin A, Lewis KL, Reizis B. Continuous expression of the transcription factor e2-2 maintains the cell fate of mature plasmacytoid dendritic cells. *Immunity* (2010) **33**:905–16. doi:10.1016/j.immuni.2010.11.023
88. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med* (2007) **204**:1765–74. doi:10.1084/jem.20070719
89. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* (2007) **204**:1757–64. doi:10.1084/jem.20070590
90. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* (2007) **204**:1775–85. doi:10.1084/jem.20070602
91. Guilliams M, Crozat K, Henri S, Tamoutounour S, Grenot P, Devilard E, et al. Skin-draining lymph nodes contain dermis-derived CD103(-) dendritic cells that constitutively produce retinoic acid and induce Foxp3(+) regulatory T cells. *Blood* (2010) **115**:1958–68. doi:10.1182/blood-2009-09-245274
92. Gatto D, Wood K, Caminschi I, Murphy-Durland D, Schofield P, Christ D, et al. The chemotactic receptor EBI2 regulates the homeostasis, localization and immunological function of splenic dendritic cells. *Nat Immunol* (2013) **14**:446–53. doi:10.1038/ni.2555
93. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* (2011) **35**:780–91. doi:10.1016/j.immuni.2011.08.013
94. Satpathy AT, Briseno CG, Lee JS, Ng D, Manieri NA, Kc W, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol* (2013) **14**:937–48. doi:10.1038/ni.2679
95. Tussiwand R, Everts B, Grajales-Reyes GE, Kretzer NM, Iwata A, Bagaitkar J, et al. Klf4 expression in conventional dendritic cells is required for T helper 2 cell responses. *Immunity* (2015) **42**:916–28. doi:10.1016/j.immuni.2015.04.017
96. Gao Y, Nish SA, Jiang R, Hou L, Licona-Limon P, Weinstein JS, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* (2013) **39**:722–32. doi:10.1016/j.immuni.2013.08.028
97. Chou DB, Sworder B, Bouladoux N, Roy CN, Uchida AM, Grigg M, et al. Stromal-derived IL-6 alters the balance of myeloerythroid progenitors during *Toxoplasma gondii* infection. *J Leukoc Biol* (2012) **92**:123–31. doi:10.1189/jlb.1011527
98. Chorro L, Sarde A, Li M, Woollard KJ, Chambon P, Malissen B, et al. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. *J Exp Med* (2009) **206**:3089–100. doi:10.1084/jem.20091586
99. Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science* (2014) **343**:776–9. doi:10.1126/science.1247651
100. Zigmond E, Samia-Grinberg S, Pasmanik-Chor M, Brazowski E, Shibolet O, Halpern Z, et al. Infiltrating monocyte-derived macrophages and resident Kupffer cells display different ontogeny and functions in acute liver injury. *J Immunol* (2014) **193**(1):344–53. doi:10.4049/jimmunol.1400574
101. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* (2007) **10**:1538–43. doi:10.1038/nn2014
102. Ajami B, Bennett JL, Krieger C, Mcnagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci* (2011) **14**:1142–9. doi:10.1038/nn.2887
103. Yamasaki R, Lu H, Butovsky O, Ohno N, Rietsch AM, Cialic R, et al. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med* (2014) **211**:1533–49. doi:10.1084/jem.20132477
104. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**:1118–28. doi:10.1038/ni.2419
105. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
106. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* (2014) **41**:14–20. doi:10.1016/j.immuni.2014.06.008
107. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* (2015) **42**:419–30. doi:10.1016/j.immuni.2015.02.005
108. Siddiqui KR, Laffont S, Powrie F. E-cadherin marks a subset of inflammatory dendritic cells that promote T cell-mediated colitis. *Immunity* (2010) **32**:557–67. doi:10.1016/j.immuni.2010.03.017
109. Guilliams M, Movahedi K, Bosschaerts T, Vandendriessche T, Chuah MK, Herin M, et al. IL-10 dampens TNF/inducible nitric oxide synthase-producing dendritic cell-mediated pathogenicity during parasitic infection. *J Immunol* (2009) **182**:1107–18. doi:10.4049/jimmunol.182.2.1107
110. Kuipers H, Soullie T, Hammad H, Willart M, Kool M, Hijdra D, et al. Sensitization by intratracheally injected dendritic cells is independent of antigen presentation by host antigen-presenting cells. *J Leukoc Biol* (2009) **85**:64–70. doi:10.1189/jlb.0807519
111. Cheong C, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP, et al. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* (2010) **143**:416–29. doi:10.1016/j.cell.2010.09.039
112. Shalek AK, Satija R, Adiconis X, Gertner RS, Gaublomme JT, Raychowdhury R, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. *Nature* (2013) **498**:236–40. doi:10.1038/nature12172

113. Ohl L, Mohaupt M, Czeloth N, Hintzen G, Kiafard Z, Zwirner J, et al. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. *Immunity* (2004) **21**:279–88. doi:10.1016/j.immuni.2004.06.014
114. Jang MH, Sougawa N, Tanaka T, Hirata T, Hiroi T, Tohya K, et al. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. *J Immunol* (2006) **176**:803–10. doi:10.4049/jimmunol.176.2.803
115. Guilliams M, Malissen B. A death notice for in-vitro-generated GM-CSF dendritic cells? *Immunity* (2015) **42**:988–90. doi:10.1016/j.immuni.2015.05.020
116. Helft J, Bottcher J, Chakravarty P, Zelenay S, Huotari J, Schraml BU, et al. GM-CSF mouse bone marrow cultures comprise a heterogeneous population of CD11c(+)MHCII(+) macrophages and dendritic cells. *Immunity* (2015) **42**:1197–211. doi:10.1016/j.immuni.2015.05.018
117. Wimmers F, Schreibelt G, Skold AE, Figdor CG, De Vries IJ. Paradigm shift in dendritic cell-based immunotherapy: from in vitro generated monocyte-derived DCs to naturally circulating DC subsets. *Front Immunol* (2014) **5**:165. doi:10.3389/fimmu.2014.00165
118. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen JL, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**:1261–71. doi:10.1084/jem.20092618
119. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) **15**:929–37. doi:10.1038/ni.2967
120. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* (2014) **40**:91–104. doi:10.1016/j.immuni.2013.11.019
121. Molawi K, Wolf Y, Kandalla PK, Favret J, Hagemeyer N, Frenzel K, et al. Progressive replacement of embryo-derived cardiac macrophages with age. *J Exp Med* (2014) **211**:2151–8. doi:10.1084/jem.20140639
122. Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science* (2009) **325**:612–6. doi:10.1126/science.1175202
123. Schraml BU, Van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* (2013) **154**:843–58. doi:10.1016/j.cell.2013.07.014
124. Poltorak MP, Schraml BU. Fate mapping of dendritic cells. *Front Immunol* (2015) **6**:199. doi:10.3389/fimmu.2015.00199

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Ontogeny of tissue-resident macrophages

Guillaume Hoeffel and Florent Ginhoux**

*Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore*

The origin of tissue-resident macrophages, crucial for homeostasis and immunity, has remained controversial until recently. Originally described as part of the mononuclear phagocyte system, macrophages were long thought to derive solely from adult blood circulating monocytes. However, accumulating evidence now shows that certain macrophage populations are in fact independent from monocyte and even from adult bone marrow hematopoiesis. These tissue-resident macrophages derive from sequential seeding of tissues by two precursors during embryonic development. Primitive macrophages generated in the yolk sac (YS) from early erythro-myeloid progenitors (EMPs), independently of the transcription factor c-Myb and bypassing monocytic intermediates, first give rise to microglia. Later, fetal monocytes, generated from c-Myb$^+$ EMPs that initially seed the fetal liver (FL), then give rise to the majority of other adult macrophages. Thus, hematopoietic stem cell-independent embryonic precursors transiently present in the YS and the FL give rise to long-lasting self-renewing macrophage populations.

Keywords: macrophages, monocytes, fetal liver, yolk sac, C-Myb, erythro-myeloid progenitors, hematopoiesis, hematopoietic stem cells

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****Correspondence:***
*Guillaume Hoeffel and Florent Ginhoux, Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), 8A Biomedical Grove, IMMUNOS Building #3-4, Biopolis, 138648 Singapore*
guillaumehoeffel1@gmail.com; florent_ginhoux@immunol.a-star.edu.sg

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Introduction

Ilya (Elie) Metchnikoff first described the mechanism of phagocytosis and the cells responsible for this process over a century ago. These professional phagocytic cells were named "macrophages" (from the Greek derivation macro = large and phage = devouring, "large devouring cells"). These were separate from "microphages," which included polymorphonuclear phagocytes (1). Determining the role of macrophages in pathogenic infections was one of the fundamental observations leading to the concept of cellular immunity (2). Through this seminal work, Metchnikoff anticipated the central role of macrophages in tissue inflammation and homeostasis. We recommend an elegant historical review for more details about Metchnikoff's work by Yona and Gordon in this issue (3).

Since then, the definition of the phagocyte system has been continuously refined, and our understanding of the wide-ranging functions of macrophages has been substantially expanded. It is now clear that, in addition to their classical function in the activation and resolution of tissue inflammation, macrophages also play roles in tissue-specific functions, tissue remodeling during angiogenesis and organogenesis, and wound healing, to name a few (4). Macrophages are exquisitely adapted to their local environment, acquiring organ-specific functionalities during developmental stages and the steady state (4). Macrophages are able to support multiple tissue functions, integrating cues from both the outside environment and their microenvironment to act as rheostatic cells of tissue function. Thus, tissue-resident macrophages represent an attractive target for modern medicine to treat a wide spectrum of diseases in which they have been implicated, including atherosclerosis, autoimmune diseases, neurodegenerative and metabolic disorders, and tumor growth (5–8). Understanding the

origin and developmental pathways of macrophages will help to design novel intervention strategies targeting these cells in tissue-specific sites.

A number of observations now indicate that certain macrophage populations derive from embryonic precursors sequentially seeding tissues during development (9–13). Two macrophage progenitors, yolk sac (YS) macrophages and fetal monocytes, have been described in the embryo, but their exact nature and origin were not fully understood until recently (14, 15). Here, we discuss recent developments in our understanding of the origin of adult tissue-resident macrophages, exploring the sequence of progenitors generated during embryonic and adult hematopoiesis. We focus on the relative contributions of YS macrophages and fetal or adult monocytes, including a discussion of our own recent data exploring the heterogeneity of fetal monocyte developmental pathways.

Early Concepts

Macrophages form part of the mononuclear phagocyte system (MPS), which also includes circulating monocytes and dendritic cells (16). Until recently, our vision of macrophage origin and homeostasis was largely based on seminal studies that used *in vivo* radioisotope labeling and radiation chimera experiments. These studies led to the early dogma that resident macrophages were constantly replenished from circulating bone marrow (BM)-derived monocytes as a continuum of differentiation (17–19).

In agreement with that concept, studying the ontogeny of the MPS revealed that monocytes and macrophages derived from macrophage and dendritic cell progenitors (MDPs) present in the BM, which are phenotypically defined as lineage-c-kit$^+$CX 3CR1$^+$Flt3$^+$CD115$^+$ (20). MDPs further differentiate through a newly described common monocyte precursor (cMoP), phenotypically defined as lineage$^-$c-kit$^+$CX3CR1$^+$Flt3$^-$CD115$^+$ (21), that gives rise to the two main subsets of circulating monocytes distinguished by the expression of Ly6C (22).

Specific tissue macrophages, such as dermal, gut, and heart macrophages, seemed to follow the model of Van Furth, that macrophages are derived from monocytes (23–25). However, this model did not fit in all cases and evidence also emerged indicating that macrophages were long-lived cells, able to self-renew locally. Hashimoto was the first to speculate that Langerhans cells (LCs) represented a self-perpetuating "intraepithelial phagocytic system" (26). Performing a human skin transplantation assay onto nude mice, Krueger et al. described the remarkable longevity of LCs, which were able to persist in the grafts for more than 2 months (27). Their ability to self-renew through proliferation was later described using DNA densitometry (28). Similar conclusions were drawn soon after regarding alveolar macrophages (29). The dominant concept of "the monocytic origin" of tissue macrophages was also challenged through experiments in animals with prolonged monocytopenia following strontium-89 monocyte depletion, in which liver Kupffer cells were shown to maintain cell numbers by increasing local proliferation (30, 31).

More recently, the use of long-term parabiotic mice and subsequent fate-mapping models have challenged the MPS paradigm and revealed that, unlike all other hematopoietic cells, which rely on hematopoietic stem cell (HSC)-derived BM progenitors, certain macrophage populations possess the unique ability to self-renew locally independently of circulating precursors (32–36). Initial studies describing the presence of macrophages in embryonic tissues suggested that tissue macrophages derived from embryonic progenitors. In rodents, macrophage-like cells first described in the brain rudiment and in the developing skin (37, 38) were named "fetal macrophages" and found to exhibit a high capacity for proliferation (39). These observations suggested that adult macrophages derive from fetal macrophages established during early development. However, whether these fetal macrophages were maintained until adulthood or were replaced postnatally was not addressed until recently. In addition, the exact nature and the origin of fetal macrophage progenitors remained unclear.

Embryonic Hematopoiesis

Mammalian embryos produce several transient waves of hematopoietic cells before the establishment of HSCs in the BM during late gestation (40, 41). The multiple embryonic waves are differentially regulated in time and space and exhibit distinct lineage potentials. Importantly, they contribute to hematopoietic populations that persist until adulthood. These waves include primitive hematopoiesis in the YS, and definitive hematopoiesis, which comprises a transient definitive stage, generating multi-lineage erythro-myeloid progenitors (EMPs) and lympho-myeloid progenitors (LMPs), and a definitive stage characterized by the production of HSCs in the aorta-gonad-mesonephros (AGM). These transient progenitors establish themselves transiently in the fetal liver (FL) during the mid to late stages of hematopoiesis. The sequential waves of hematopoiesis can overlap in time and space (**Figure 1**) and remain difficult to separate clearly, even with the most recent fate-mapping tools available.

Primitive Hematopoiesis

In mice, the first hematopoietic progenitors appear in the extra-embryonic YS blood islands at around embryonic age 7.25 (E7.25), where primitive hematopoiesis is initiated, producing mainly nucleated erythrocytes. This observation linked the myeloid progenitors observed in the YS at E7 with the emergence of YS macrophages after E9.0 [**Figure 2**; Ref. (42–45)]. Primitive hematopoiesis was also shown to produce megakaryocyte progenitors (46). The denomination "primitive" was given to reflect the production of embryonic erythroblasts, like those observed in lower species such as fish, amphibians, and birds, and remaining nucleated throughout their life span (47–49). This denomination was extended to macrophages in the YS due to their concomitant development prior to FL hematopoiesis. Interestingly, no clear evidence of monocytic intermediates was reported at this stage, although the seminal study of Cline and Moore did mention the existence of local intermediate cells between progenitors and functional macrophages (43). Studies by Naito and Takahashi et al. clarified the emergence of primitive macrophages in the YS blood islands in the mouse and rat, observing an absence of endogenous peroxidase activity as a surrogate marker for an absence of monocytic intermediates, such as those found in the

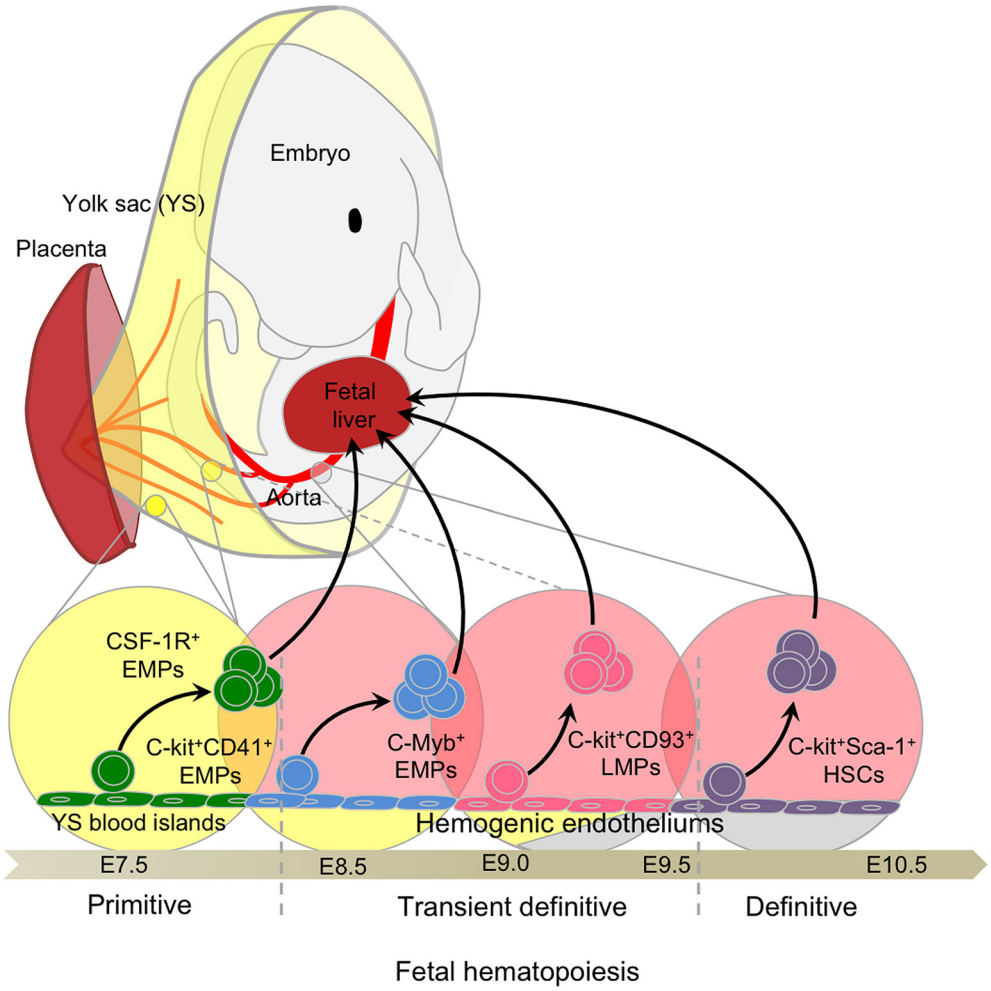

FIGURE 1 | Fetal hematopoiesis. Primitive, transient definitive, and definitive waves of fetal hematopoiesis sequentially generate progenitors able to seed the fetal liver. Primitive hematopoiesis starts at E7.0 in the blood islands of the extra-embryonic yolk sac (YS) and generates erythro-myeloid progenitors (EMPs). Early EMPs initially express CD41 and later, CSF-1R, a signature of myeloid/macrophage commitment. Concomitant to the establishment of the blood circulation at E8.5, the YS hemogenic endothelium (HE) generates late EMPs expressing C-Myb. At approximately E9.0, the intra-embryonic mesoderm generates additional HE and emerging progenitors with lymphoid potentials (LMPs) without long-term reconstitution (LTR) capacity. These C-Myb$^+$ EMPs and LMPs constitute the so-called transient definitive wave. Finally, hematopoietic stem cells (HSCs) with LTR activity emerge from the main HE situated in the aorta-gonad-mesonephros (AGM) regions and in the placenta.

BM (50–52), suggesting a unique developmental pathway for YS macrophages (53, 54).

Transient Definitive Hematopoiesis

The quest to elucidate the origins of embryonic HSCs led to the discovery of earlier lineage-restricted HSC-independent progenitors seeding the FL at E10.5. These progenitors arise concurrently with the transition of primitive to definitive erythropoiesis and were thus considered to form a transient stage of definitive hematopoiesis (45, 47, 55). Transient definitive hematopoiesis consists of progenitors sequentially acquiring myeloid, then lymphoid potential, without exhibiting the long-term reconstitution potential of HSCs. Seminal work from Palis and colleagues on embryonic erythropoiesis in the YS described the parallel emergence of multiple myeloid lineage potential progenitors from E8.25 in the YS (45). Palis et al. first observed the emergence of definitive progenitors for mast cells and a bipotential granulocyte/macrophage progenitor. These progenitors then migrated to the FL through the bloodstream after E8.5, once circulation was established (44, 56). From this pattern of development, the authors concluded that definitive hematopoietic progenitors arise in the YS, migrate through the bloodstream, and seed the FL to rapidly initiate the first phase of intra-embryonic hematopoiesis. Similarly, primitive and definitive erythropoiesis, associated with myelopoiesis, was also shown to emerge prior to HSC in the zebrafish embryo (57). Bertrand et al. showed that definitive hematopoiesis initiates in the posterior blood island with only transient proliferative potential. Because these HSC-independent definitive progenitors were observed to produce definitive erythroid and myeloid

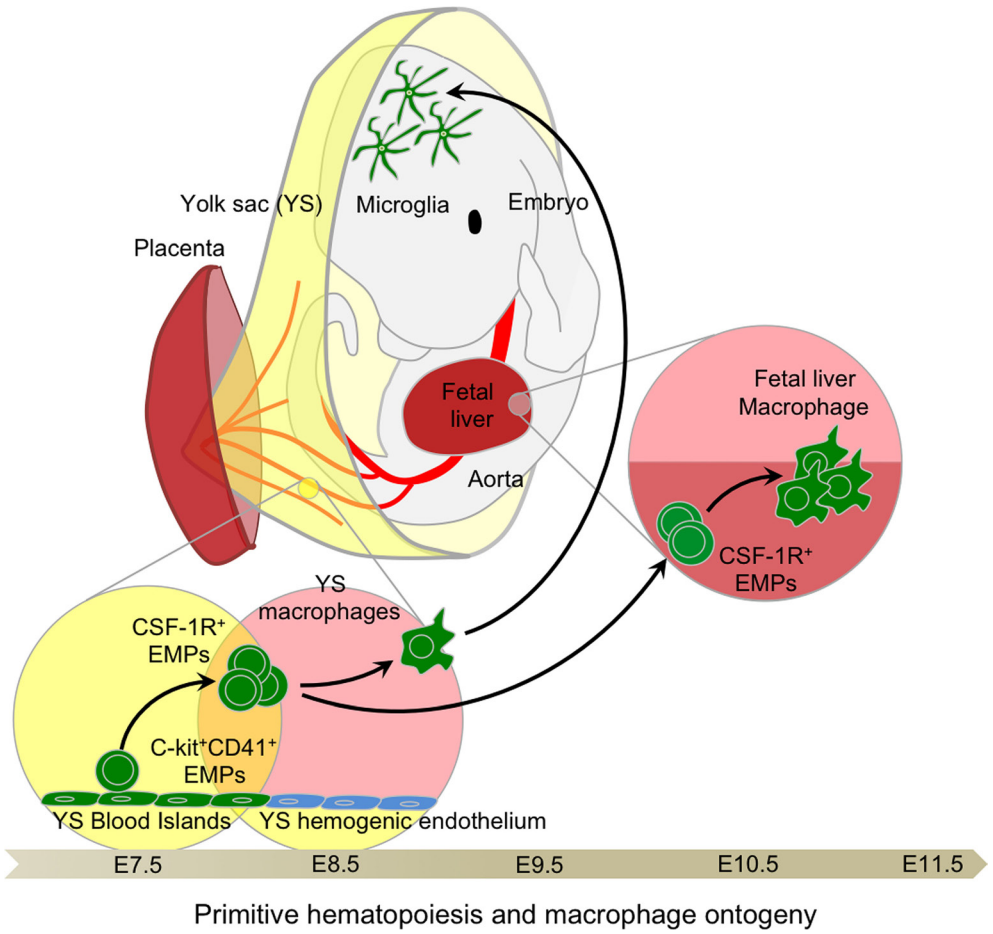

FIGURE 2 | Primitive hematopoiesis and yolk sac macrophage ontogeny. Early EMPs emerge in the YS around E7.5 before establishment of the blood circulation. They express CD41 and CSF-1R and are independent of the transcription factor C-Myb. Upon establishment of the blood circulation around E8.5, EMPs differentiate into primitive macrophages as well as primitive erythrocytes and granulocytes. Primitive macrophages seed all fetal tissues, in particular the head where they will give rise to future brain microglia that are able to continuously self-renew throughout adulthood. EMPs seeding the fetal liver briefly expand to generate a local macrophage population, likely important for sustaining enucleation of primitive erythrocytes passing through the sinusoid prior to the establishment of definitive hematopoiesis and the generation of fetal monocyte-derived macrophages in the fetal liver.

cell types, but not to colonize the zebrafish thymus (implying that they are devoid of lymphoid potential), this population was termed EMPs (57). Interestingly, EMPs can also emerge from the hemogenic endothelium (HE) located in the placenta and umbilical cord (58) and colonize the FL from E9.5 (55) to participate in definitive hematopoiesis. Further studies advanced the field significantly by identifying CD41 as an early marker (pre-CD45) for defining hematopoietic progenitors, including EMPs, emerging from the YS (59, 60). Altogether, these important studies provided phenotypic and functional analyses of the first hematopoietic progenitors and demonstrated that definitive hematopoiesis proceeds through two distinct waves during embryonic development (**Figure 3**).

In parallel, several groups have also identified other multipotential progenitors with lymphoid- or myelo-lymphoid-restricted potential in the YS and the developing para-aortic splanchnopleura (P-Sp) prior to HSCs (61, 62). Lacaud et al. also described AA4.1 $(CD93)^+$ multipotential progenitors present in the E14.5 FL with T cell, B cell, and macrophage potential (63), although the precise origin of these progenitors was not addressed. At the same time, the team of Jacobssen identified $Flt3^+$ lympho-myeloid progenitors (LMPs) devoid of erythrocyte and megakaryocyte capacity (64). Later, cells with myelo-erythroid and lymphoid lineage potential, such as B-1 cells present in the adult spleen, were associated with E9.5 YS progenitors expressing AA4.1 and CD19 (65). A year later, the same team also identified T cell potential within the E9.5 YS progenitors (66). Using a Rag-1-Cre fate-mapping model, Boiers et al. confirmed that these LMPs emerged at approximately E9.5 in the YS, seeding the FL by E11.5 to give rise to T and B cells, as well as granulocytes and monocytes in the E14.5 FL, prior to HSCs (67). Finally, lympho-myeloid progenitors isolated from the dorsal aorta at E9.0 were shown to acquire long-term reconstitution capacity after a few days of *in vitro* culture with stromal cells, and were called immature HSCs (68). Without preculture, these multipotential progenitors can only engraft natural killer (NK)-deficient $Rag2\gamma c^{-/-}$ mice.

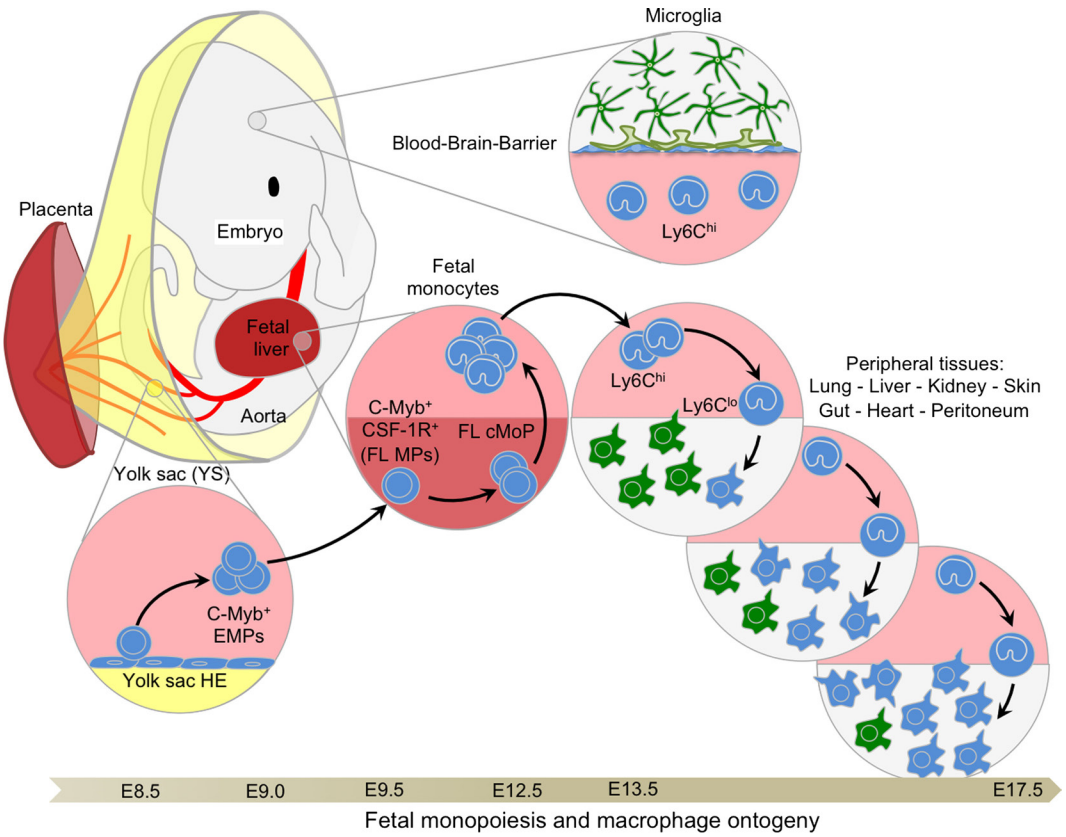

FIGURE 3 | Fetal monopoiesis and macrophage ontogeny. At E8.5 as the blood circulation is established, the YS HE, possibly in conjunction with other hemogenic sites, generates EMPs expressing CD41 and c-Myb. These late EMPs seed the fetal liver around E9.5 where they expand rapidly to give rise to CSF-1R$^+$ myeloid progenitors that are able to generate fetal monocytes through cMoP intermediates at E12.5. Fetal monocytes then spread via the blood circulation to all tissues, with the exception of the brain, which is isolated by the establishment of the blood–brain barrier at approximately E13.5. In the tissues, fetal monocytes begin to differentiate into macrophages, progressively outnumbering the previously established primitive macrophages. These fetal monocyte-derived macrophages maintain the capacity to self-renew throughout adulthood in certain tissues, such as the liver or the lung, where they will not be replaced by adult BM-derived monocytes.

Whether these immature HSCs arise from LMPs or represent a distinct wave of progenitors remains to be clarified. However, these seminal studies provided strong evidence that lymphoid potential can emerge from the YS, prior to HSC-budding from the AGM (69).

Because the emergence of EMPs and LMPs overlaps in time and space, they could not be distinguished clearly until recently. Previous reports had suggested that lymphoid potential was restricted to the CD41-negative cell fraction (59, 65). However, CD41 is also expressed in a sub-fraction of FL HSCs, and so this phenotypical distinction spread some confusion (47). Finally, a recent report from the group of Palis clarified this point by showing that co-expression of c-kit, CD41, and CD16/32 defines EMPs and allows their separation from other progenitors with lymphoid potential, such as those giving rise to the B-1 cell (70). McGrath et al. extended the notion of EMPs by showing their potential to generate neutrophils, megakaryocytes, macrophages, and erythrocytes. Finally, transplantation of EMPs in immune-compromised adult mice can also provide transient adult red blood cell reconstitution (70).

To conclude, commitment to hematopoietic fates begins during gastrulation in the YS, which represents the only site of primitive erythropoiesis and also serves as the first source of transient definitive hematopoietic progenitors. HE develops from the YS to various intra-embryonic sites, and acquires myeloid and then lymphoid lineage potentials in overlapping waves, highlighting the complexity of the hematopoietic output. Whether some of these progenitors arise from independent sources or represent different maturation stages of a shared hematopoietic wave, culminating with the generation of HSCs, needs to be further clarified. However, it is tempting to speculate that the clear contrasts in differentiation/lineage potential do not reside in their intrinsic potential, but rather in the extrinsic signals provided by the local environment.

Definitive Hematopoiesis

The complex hierarchy of stem and progenitor cells in the BM is first established during embryonic development starting with the emergence of small numbers of HSCs from the AGM at E10.5 in murine embryos or at 5 weeks in human embryos

(71, 72). After E9.5 in the mouse, with the determination of the intra-embryonic mesoderm toward a hematopoietic lineage, new waves of hematopoietic progenitors emerge within the HE of the embryo proper (**Figure 4**), first in the P-Sp region and the umbilical and vitelline arterial regions of the embryo, then in the AGM region and the placenta (55, 73, 74). The hematopoietic activities of the P-Sp and AGM first generate immature HSCs and then mature HSCs, which are defined by their capacity to reconstitute adult conventional mice (long-term reconstitution; LTR). Both immature and mature HSCs seed the FL at approximately E10.5 (68, 71, 75, 76) to establish definitive hematopoiesis (40, 77, 78). A maturation step seems necessary for immature HSCs to express their LTR activity in full, which is then maintained until adulthood (68). However, further investigations using a fate-mapping system would be necessary to confirm this model.

The FL becomes the major hematopoietic organ after E11.5, generating all hematopoietic lineages. Importantly, the FL itself does not produce progenitors *de novo*, but rather recruits progenitors derived from the YS and other hemogenic sites, to initiate definitive hematopoiesis (79) in parallel with the expansion of the definitive HSC population before their migration to the spleen and BM (80).

The contribution of HSCs to FL hematopoiesis is complex to evaluate, partly because of the lack of specific fate-mapping models, and also the relatively limited knowledge regarding embryonic HSC maintenance and homeostasis in this environment. The capacity for long-term reconstitution, which defines functional HSCs, is present in the AGM by E10.5 (76). However, lineage-specific commitment may not occur *in vivo* immediately after reaching the FL environment. A number of other progenitors generated during transient definitive hematopoiesis, as discussed above, are already present and able to give rise to almost all cell lineages, which could prevent HSC consumption and differentiation (**Figure 5**). Evaluation of HSC contribution has long been based on the assumption that all hematopoietic cells in the FL were derived from HSCs as is the case in the BM (81). Many multipotential progenitors share the same phenotype with pre-HSC and HSCs, such as the expression of CD41 and AA4.1 (60), adding to this confusion. The combination of the marker Sca-1 and new markers such as those from the SLAM family (82) have greatly helped to clarify the characterization of HSCs, defined now as Lin$^-$ckit$^+$Sca-1$^+$CD150$^+$CD48$^-$CD244$^-$. However, no specific fate-mapping model exists to characterize embryonic HSC progeny with the exception of the Flt3-Cre

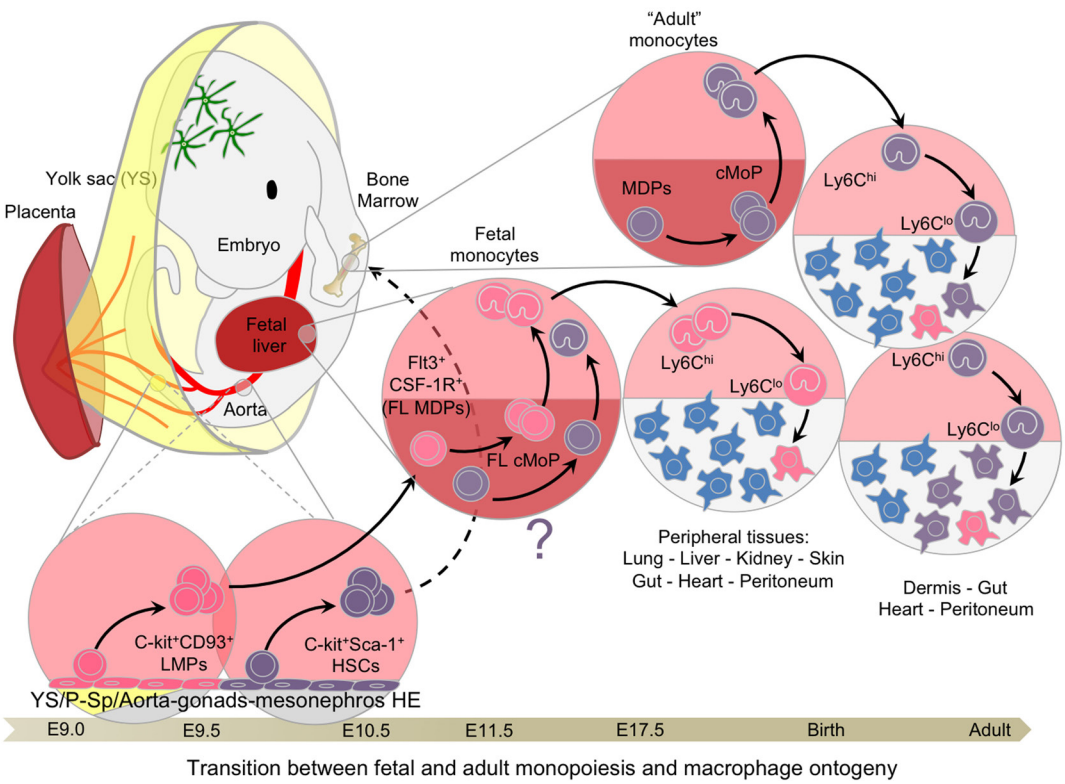

FIGURE 4 | Transition between fetal and adult hematopoiesis. Hemogenic endothelial cells from extra and intra-embryonic hematopoietic tissues generate C-Myb-dependent multipotential progenitors, such as LMPs and pre-HSCs, between E9.0 and E10.5, culminating with the emergence of mature HSCs with long-term reconstitution-bearing potential. CD93 (AA4.1) expression is associated with the emergence of lymphoid potential, whereas Sca-1 is the hallmark of HSCs. These progenitors seed the fetal liver around E10/E11, expanding and giving rise to the various lineages of the hematopoietic system, including fetal monocytes. These late fetal monocytes continue to participate in the tissue-resident macrophage network until hematopoiesis switches completely from the fetal liver to the bone marrow. Once adult hematopoiesis begins to take place in the bone marrow generating monocytes, certain tissues, such as the dermis, heart peritoneum, and the gut, continue to recruit adult monocytes to generate resident macrophages and replace with time the embryonic-derived macrophages.

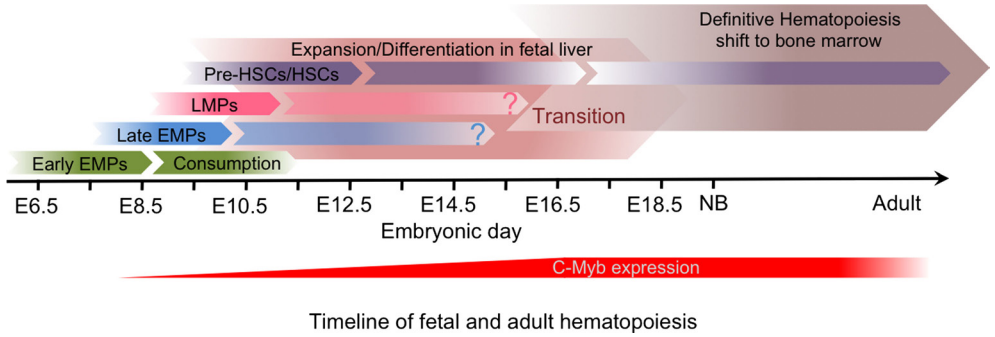

FIGURE 5 | Timeline of fetal and adult hematopoiesis. The primitive hematopoiesis is initiated in the yolk sac independently of C-Myb activity, and generates early CSF-1R$^+$ EMPs that give rise to YS macrophages without monocytic intermediates during a short time window and will establish the brain microglia. The transient definitive hematopoiesis and then the definitive hematopoiesis are both dependent on C-Myb activity and generate progenitors that differentiate in the fetal liver. The transient definitive wave, which include EMPs and then LMPs, give rise in particular to fetal monocytes that seed the tissues prior to birth to establish the self-renewing tissue-resident macrophage network. Although only HSCs, which result from the definitive hematopoiesis, seem to be maintained in the bone marrow in adults, the relative contribution of the transient definitive wave to the adult immune system remains unclear.

model (83), which was used until now with the assumption that embryonic and adult HSCs follow similar differentiation pathways. Our recent report suggests that the Flt3-Cre model can also be used to follow the progeny of LMPs (15). Furthermore, in the nascent BM, the long-term repopulation (LTR) capacity that characterizes functional HSCs is only observed at around E17.5 (84). Considering the time required to initiate full HSC differentiation, these data suggest that proper adult HSC-derived hematopoiesis does not take place in the BM until a few days after birth. Characterization of the functional specificities and regulatory pathways of HE that give rise to HSCs versus those that generate EMPs and other multipotential progenitors could aid the development of new fate-mapping models and improve our understanding of this process (85). Use of other fate-mapping models such as the Runx1-Mer-Cre-Mer (Runx1-iCre) (86), Tie2-Mer-Cre-Mer mice (14), and the c-kit-Mer-Cre-Mer mice (87) provided complementary results, although a careful analysis of the targeted cells in time and space is not yet fully available for the last two models. We present here our best interpretation of the data provided in these two recent studies that have used these models in light of the literature and our own results and experience using the Runx1-iCre model (**Figure 6**).

Embryonic and Adult Precursors of Adult Tissue-Resident Macrophages

Yolk Sac Macrophages

Yolk sac macrophages first appear in the YS blood islands at E9 (albeit in small numbers) with a unique pattern of differentiation that bypasses the monocytic intermediate stage seen in adult macrophages (50, 52). YS-derived primitive macrophages spread into the embryo proper through the blood as soon as the circulatory system is fully established (from E8.5 to E10) (56), and migrate to various tissues, including the brain. Importantly, this occurs before the onset of fetal monocyte production by the FL, which starts around E11.5/E12.5 (92). These primitive macrophages retain the high proliferative potential observed in the YS as they colonize various tissues (52, 93–95). Primitive macrophages may contribute to many fundamental processes during mid and late embryogenesis, such as clearance of dead cells or tissue maturation. In this regard, the developmental process of interdigital cell death removal during the mouse footplate remodeling that occurs between E12.5 and E14.5 is of interest as the interdigit regions become heavily populated by macrophages and most of the dead cells were shown to be rapidly engulfed by macrophages (96). However, mouse models devoid of primitive macrophages such as the colony-stimulating factor 1 receptor (CSF-1R) KO (Florent Ginhoux, unpublished data,) and PU.1 KO (97) appears to exhibit a normal interdigit web tissue. Wood et al. observed that interdigit web tissue in PU.1 KO was only slightly retarded, suggesting that other cell type such as neighboring mesenchymal cells were compensating (97). In addition, we recently showed that depletion of primitive macrophages and hence of embryonic microglia, affected the progression of dopaminergic axons in the forebrain and the laminar positioning of subsets of neocortical interneurons, likely through phagocytic mechanisms (98).

Schulz et al. highlighted further differences between primitive and definitive hematopoiesis, showing that the latter relies on the transcription factor Myb, while YS-derived macrophages are Myb-independent, and are instead dependent on PU.1 (12). This again reinforces the view that YS-derived macrophages constitute an independent lineage, distinct from the progeny of definitive HSCs. Schulz et al. exploited the differential dependence of primitive versus definitive hematopoiesis on the transcription factor c-Myb and reported that E16.5 tissue macrophage populations were not affected by the loss of c-Myb. Using a CSF-1R-iCre fate-mapping model of YS macrophages, they also reported the persistence of YS macrophages progeny in adult tissue-resident macrophage populations (lung, liver, and pancreas, as well as in the brain and skin), although the level of labeling was minimal (below 3–5%) and decreased with time. The authors concluded that tissue-resident macrophages were therefore derived from a c-Myb-independent lineage via YS macrophages (12), data supporting the initial report showing that microglia arise from

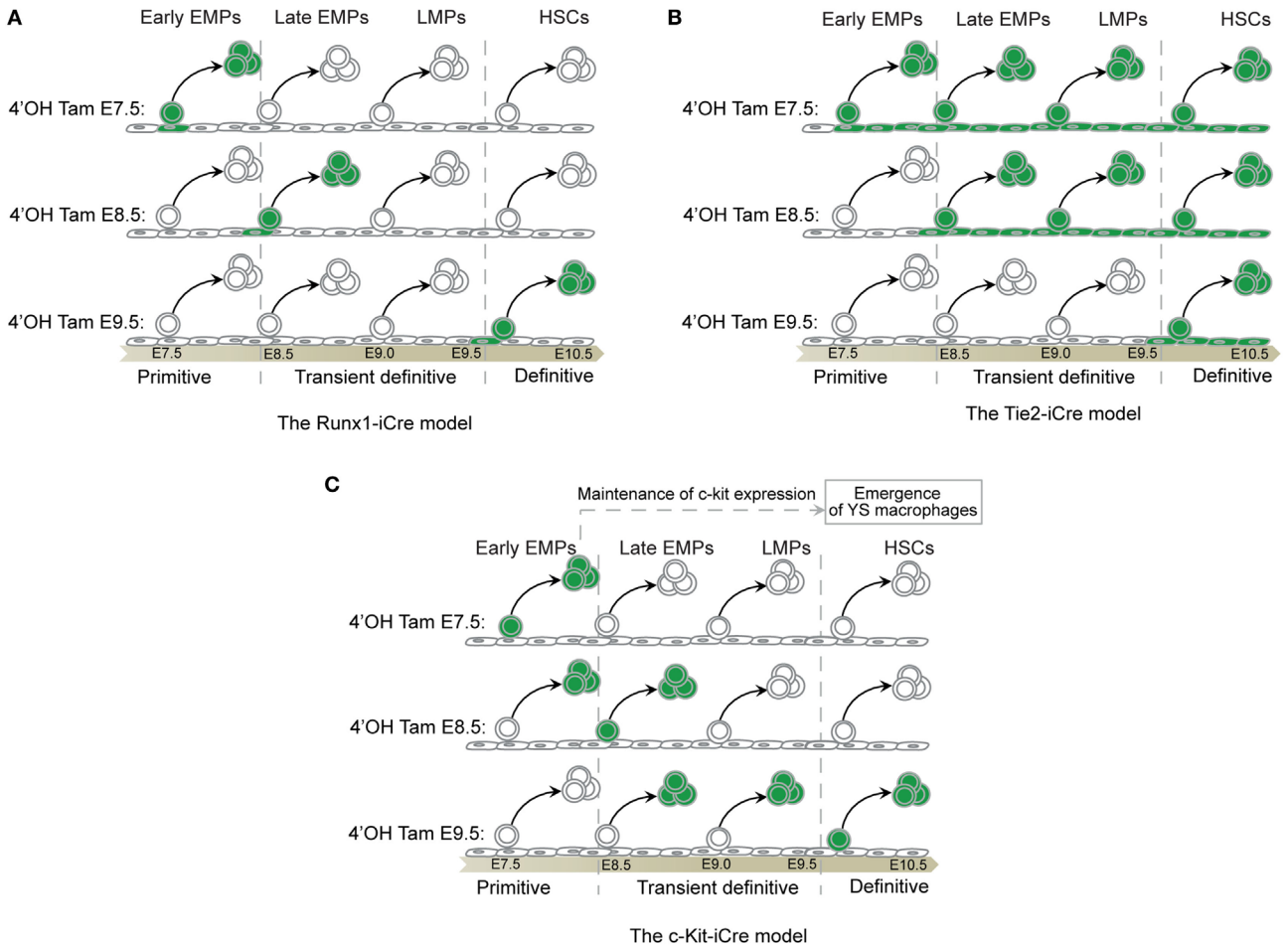

FIGURE 6 | Fate-mapping systems. **(A)** The Runx-iCre fate-mapping model (86) used in our study targets the hemogenic transition (88), hence, labeling specifically progenitors in the process of budding out from the hemogenic endothelium. The Runx1 expression decreases in progenitors once they start to express Vav thus reducing the chances of tagging released progenitors from precedent waves (88). As a consequence, Runx-iCre tagging is restricted to a short time window in the lifespan of a given progenitor and allows a sharp definition of each hematopoietic wave. However, this model also restricts the tagging to only a small fraction of the targeted progenitor wave. **(B)** Tie2 is expressed in all endothelial cells that constitute the hemogenic endothelia even before the hemogenic transition (89). Thus, all endothelial cells and their progeny (non-hematopoietic and hematopoietic cells) will be labeled after tamoxifen injection using the Tie2-iCre model. As a consequence, an early tamoxifen injection (such as at E7.5) will result in the tagging of all hematopoietic cells emerging before the time of analysis. This will include progenitors from the primitive, the transient definitive, and the definitive waves if, for example, the analysis is done at E11.5. A late injection (such as at E10.5) will restrict the tagging to only the latest hematopoietic stem cells wave as they are just budding from HEs (90). Thus, this model might not be suitable to clearly separate the primitive from the transient definitive waves of hematopoiesis. However, this model could be important to study late HSC progeny as no other progenitors than HSCs emerge from HE after E10.5 (91). **(C)** C-kit is expressed by all hematopoietic progenitors and does not label endothelial cells that constitute the HEs (89). An early tamoxifen injection (such as at E7.5) will restrict the labeling to early progenitors making suitable the c-kit-iCre model to study the primitive hematopoiesis. However, the FL recruits progenitors of each hematopoietic wave from E8.5 until E11 (79). These progenitors still express c-kit and coexist after seeding the FL during the time necessary for their differentiation (47, 55). A later tamoxifen injection (such as at E9.5) might thus result in the cumulative labeling of undifferentiated primitive and definitive progenitors, including the transient wave of EMPs and LMPs. Thus, such model may not be suitable to resolve the complexity of the different embryonic hematopoietic waves characterized by short time windows of emergence and strong overlapping tendencies. Primitive hematopoietic progenitors are rapidly consumed and the engagement of EMPs and LMPs in FL hematopoiesis reduces the expression of c-kit on their surface. Thus, later tamoxifen injection (such as at E11.5) could restrict the labeling to newly derived HSCs expressing high level of c-kit without labeling precedent progenitor waves (87). Such model might be interesting to study the progeny of late HSCs although the risk of tagging the progeny of EMPs and LMPs or later committed progenitors derived from HSCs remains high and difficult to exclude. Further analysis would be necessary to clarify the potential of such model.

YS macrophages (9). Embryonic origin of macrophages was further supported by the work of Yona et al. and Hashimoto et al. showing that adult monocytes do not substantially contribute to tissue macrophages under steady-state conditions (13, 33). Furthermore, Yona et al. suggested the existence of a CX3CR1^{+} precursor for some of the monocyte-independent macrophages, although the exact nature of this precursor was not elucidated. In fact, both YS macrophages and fetal monocytes express CX3CR1 (9, 11, 15) and could therefore correspond to the unidentified precursors suggested by Yona et al. However, using a CSF-1R-iCre fate-mapping model, also used by Schulz et al. (12), another study noted that the YS macrophage contribution in the brain,

the adult liver, and the heart was maintained although at a minimal level that decreased with time (99). Interestingly, the level of labeling was always higher in microglia than that in the liver or the heart, suggesting that the level of YS macrophage contribution may differ between tissues and that YS macrophages may be differently replaced over time by later waves of progenitors, which follow tissue-distinct kinetics (discussed below). Our own report using the Runx1-iCre fate-mapping model (86) indicated that only microglia, specialized macrophages of the central nervous system, were derived solely from primitive macrophages while all other tissue macrophages derived from definitive hematopoiesis (9).

To understand whether YS macrophages might be the sole progenitors of every other adult macrophages, we asked what impact their *in utero* depletion would have on the subsequent generation of fetal tissue macrophages. CSF-1R is expressed on YS macrophages and fetal monocytes, but only the development of the former is actively dependent on CSF-1R (9, 11). Thus, we attempted to deplete YS macrophages by transiently inhibiting the CSF-1R signaling pathway using a blocking anti-CSF-1R antibody, as recently described (98). Importantly, after complete depletion of primitive YS macrophages in E10.5 embryos and thus of most macrophages in treated embryos at E14.5, tissue macrophages (including microglia) were able to repopulate to normal levels before birth. These data suggest that YS macrophages are dispensable for the generation of tissue-resident macrophages in the embryo, and that another CSF-1R-independent embryonic precursor can functionally replace YS macrophages during development (15, 98). Using a combination of both the CSF-1R-iCre and the Runx1-iCre fate-mapping models, we noted that although YS macrophages infiltrate all tissues (including lung, liver, kidney, skin, gut, heart, pancreas, and stomach) until E13.5, a second wave of precursors, with a monocytic morphology and phenotype, supersedes them after E14.5 with the exception of the brain where YS macrophages are maintained until adulthood (15). A fuller understanding of this process may help to resolve some of the earlier discrepancies regarding the contribution of YS macrophages.

Fetal Monocytes

Fetal monocytes were described by Naito et al. (92). Focusing their study on liver Kupffer cells (the resident macrophages of the liver) during embryonic development, they exploited the endogenous peroxidase activity of monocytes and pro-monocytes granules described earlier by van Furth et al. (18, 92). Naito et al. observed the transient appearance of peroxidase activity, a signature for monocyte and pro-monocyte granule activity, during the *in vitro* generation of macrophages from a preparation of FL-dissociated cells (92). In the YS and at early stages of FL development, no peroxidase activity was observed, suggesting that primitive macrophages first seed the FL. At a later stage, the peroxidase activity increased, suggesting the presence of monocytic intermediates. *In vitro* clonal expansion assays confirmed the existence of two types of colonies, those containing fetal monocytes and those devoid of them. This provided early evidence for the existence of two distinct developmental pathways leading to the generation of Kupffer cells, although at this stage, direct differentiation of fetal monocytes into macrophages *in vivo* had not been demonstrated (100).

To investigate the developmental event leading to the emergence of tissue-specific macrophages, we initially focused on the LC, the specialized myeloid population of the epidermis. While YS macrophages seed the embryonic skin before E13.5, we discovered that the major fraction of adult LCs is in fact derived from fetal monocytes that are generated in the FL from E12.5 and are then recruited into fetal skin at E14.5 (11). These cells share a similar phenotype to their adult counterparts; however, they are generated independently of CSF-1R expression (9, 11). They possess high proliferative potential, and, in contrast to their adult counterparts, express few genes related to pathogen recognition and immune activation (15). Further studies should clarify whether such differences reflect monocyte immaturity imposed by a sterile fetal environment, or rather dedicated functional specializations that have yet to be unraveled. *In utero* adoptive transfers combined with fate-mapping studies unequivocally confirmed *in situ* differentiation of fetal monocytes into adult LCs (11). Fetal monocytes were then demonstrated to be the precursor of adult macrophages in lung alveoli by intranasal injection (10, 101). Fetal monocytes were also shown to be involved in the generation of adult macrophages of the heart (99). In fact, fetal monocytes become the major leukocyte within the blood circulation after E13.5, spreading to all tissues. This occurred independently of the CCL2/CCR2 axis (15), suggesting an alternative mechanism of exit from the FL and/or recruitment by fetal tissues. Moreover, we were able to fate-map, from before birth to adulthood, the local differentiation of fetal monocytes into resident macrophages, by taking advantage of the specific expression of S100a4 in fetal monocytes compared to YS macrophages (15). Only the brain remained free from fetal monocyte infiltration, possibly resulting from the isolation of the brain by the nascent blood–brain barrier as early as E13.5 (15, 102). Thus, these data now reveal that fetal monocytes are the major circulating embryonic precursor for all macrophages, with the exception of the brain. The absence of monocyte precursor contribution to the microglial pool could result from a lack of intrinsic potential or a lack of access to the developing brain due to the nascent blood–brain barrier. Interestingly, we observed a major influx of monocytes in the brain at E14.5 in our YS macrophage depletion model, and preliminary data using our fetal monocyte S100a4-Cre/WT fate-mapping model combined with *in utero* depletion of YS macrophages suggest that fetal monocytes are capable of giving rise to microglia under certain conditions (Hoeffel & Ginhoux, personal communication). Whether this atypical fetal monocyte infiltration reflects a compensatory mechanism to fulfill an empty niche in the brain or results from a disruption of the blood–brain barrier remains to be investigated.

Adult Monocytes

BM-derived circulating monocytes were considered the only precursors for all tissue-resident macrophages since the seminal work of van Furth et al. (17–18). Although this dogma was entirely revisited recently with the emergence of sophisticated fate-mapping tools as well as parabiotic models, the physiological contribution of circulating adult monocytes to the adult

macrophage network remains valid at least in certain tissues. The continuous recruitment of circulating monocytes to the dermis has been shown to shape the adult dermal macrophage network (25). Although this study did not employ fate-mapping techniques, Tamoutounour's data suggest the existence in the dermis of both a prenatal pool of macrophages and a second pool derived from adult blood monocytes. The authors argue that the dermis, in contrast to the epidermis, continues to recruit circulating monocytes in adulthood, most likely facilitated by its high level of vascularization. The macrophage network in the intestine follows a similar model. Data from Bain et al. suggest that embryonic macrophages do not persist in adulthood in the gut, and are replaced constantly by circulating adult monocytes (23), convincingly showing that adult monocytes are the source of intestine-resident macrophages. The role of commensal microbiota in this process is supported by the observation that the use of germ-free animals or treatment with broad-spectrum antibiotics results in a significant reduction in the recruitment of Ly6C$^+$ monocytes to the colon (23). The macrophage network of the heart has also been shown to contain a component of YS macrophages and fetal monocyte-derived macrophages, both of which are maintained in adulthood (99). However, similar to the dermis and the gut, adult monocytes seem to replace embryonic macrophages progressively over time (24). The decreasing capacity for self-renewal of embryonic macrophages with age observed by Molawi et al. may explain the requirement for continuous recruitment of monocyte-derived macrophages to the heart in the absence of inflammation. It remains to be clarified whether this phenomenon occurs in other tissues as a result of aging. In agreement, proliferation of YS macrophages and fetal monocytes is very high during development (20–40% before E14.5) but decreases progressively to 10% few days after birth in most tissues and decreases to almost undetectable levels in adults (15). Interestingly, macrophage turnover seems different from one tissue to another. Following BrdU incorporation at steady state, almost no proliferation was observed in adult gut macrophages (23), while 2–5% was measured in adult heart macrophages (24). Macrophage proliferation activity can also be mobilized upon inflammation. For example, peritoneal macrophages can increase their proliferation rate from 1 to 9% in response to parasite infection or in response to IL-4 stimulation (35), while enhanced local proliferation of macrophages in atherosclerotic lesions sustain disease progression (103). The characterization of local signals regulating macrophage proliferation as well as the presence of specialized tissue niches that sustain macrophage survival, proliferation, or even "stemness" will be fundamental to better understand their tissue homeostasis.

The macrophage network of the lymphoid system seems to follow a similar pattern than in the gut and dermis. Although the lymph nodes (LN) start to develop very early in the embryo (104), they become functionally active only within the first week after birth recruiting and organizing B and T cell areas when follicles start to shape with connections to afferent lymphatics via the subcapsular sinus (105). Although macrophages are known to participate in lymphangiogenesis during development, notably by the production of VEGF (106, 107), the precise origin of the different LN macrophage populations remain poorly understood (108). The high level of foreign antigens passing through the LN during the lifespan, support the model of a constant replenishment of the local macrophage pool by circulating adult monocytes. However, the work of Jakubzick et al. suggests otherwise as tissue-patrolling monocytes at steady state seem to enter the LN without any sign of local differentiation to macrophages or dendritic cells (34). Further studies using fate-mapping systems should be addressed to clarify this point. Spleen macrophages are generated prenatally (13, 33). However, red pulp macrophages and marginal zone macrophages seem highly dependent, respectively, on the transcription factor SPI-C (109) and on the nuclear receptor LXR (110), also expressed by circulating monocytes and suggest again that embryonic-derived macrophages are replaced over time by adult monocytes-derived macrophages. The use of the S100a4-Cre fate-mapping model in our hands supports these observations and similar conclusions were obtained for BM and peritoneal macrophages (15). Although tissue microenvironment shapes certain macrophage functional specificities (111), through an ontogenic point of view, the composition of each tissue-resident macrophage pool evolves throughout life and the respective origins of each macrophage population may account for some of their key functions and cellular behaviors in a given tissue. Hence, a new challenge is to understand if an embryonic or adult origin matters for the function and the activation states of tissue-resident macrophages.

Origin and Development of YS Macrophages and Fetal Monocytes

Origin of YS Macrophages

Bertrand et al., in line with the seminal work of Palis (45), described two sequential myeloid waves within the early YS (42). Using an *in vitro* culture reporter system, Bertrand et al. observed a first wave of monopotent progenitors that gave rise only to macrophages, followed by a second wave that gave rise to a mix of granulocytes, monocytes, and macrophages. More recently, Kierdorf et al. revisited the work of Bertrand et al. exploiting organotypic embryonic brain slices to demonstrate that microglial cells derived from YS EMPs (112). Kierdorf et al. also showed that these EMPs did not express the transcription factor c-Myb, associating them with the progenitors reported by Schulz et al. (12), although a direct link with the generation of microglia *in vivo* in adulthood was not conclusively demonstrated. More recently, Perdiguero et al. used the CSF-1R-iCre fate-mapping model to show that YS macrophages are derived from CSF-1R$^+$ EMPs (14). Hence, these two studies suggest that YS macrophages, and thus microglia, would originate from c-Myb-independent CSF-1R$^+$ EMPs. Furthermore, Perdiguero et al. demonstrated that CSF-1R$^+$ EMPs were able to seed the FL by E10.5, suggesting that these progenitors could later populate other tissue niches and produce YS-like macrophage later during development in others tissues. Nevertheless, these data do not explain the low percentage of labeled adult macrophages observed by Schulz et al. using the CSF-1R-iCre fate-mapping model (12). Later observations by Epelman et al. (99), and more recently by our group using the same fate-mapping model (15), indicated that the ability of CSF-1R$^+$ EMP to reach the FL could explain

the surprising maintenance of primitive macrophages until E16.5 in c-Myb null embryos, where primitive macrophages generated in the YS as well as in the FL would be able to fulfill the empty niche left by the absence of c-Myb-dependent myeloid cells, that include fetal monocytes. However, this may not reflect the physiological situation and may instead result from a compensatory mechanism to ensure the presence of macrophages in all tissues in the absence of c-Myb activity and fetal monocytes. Using the same CSF-1R-iCre fate-mapping model (15), we were able to follow the maintenance of microglia in the brain by self-renewal from E10.5 until adulthood, linking them with CSF-1R$^+$ EMPs and confirming the previous observations of Perdiguero et al. (14). However, for all other macrophage populations, the reduction of fate-mapping reporter labeling after E13.5 confirmed the progressive replacement of YS macrophages by another unlabeled precursor arising from a different hematopoietic wave.

We previously showed that Runx1$^+$ YS progenitors that emerged at E7.5 give rise to YS macrophages and microglia (9, 11). Using both the Runx1-iCre and the CSF-1R-iCre fate-mapping models, we showed that these E7.5 Runx1$^+$ YS progenitors were in fact the same CSF-1R$^+$ EMPs described by Perdiguero et al. and Kierdorf et al., which contributed to the generation of YS macrophages and, to a lesser extent, those seeding the FL (14, 15, 112). However, we also observed their disappearance from the FL after E11.5 indicative of a rapid local consumption/differentiation rather than long-term maintenance. Our results also suggest that these early CSF-1R$^+$ EMPs are able to contribute to a short-term maintenance of macrophages in the FL (**Figure 2**), but do not contribute to other tissue macrophages as evidenced by their rapid disappearance from the blood circulation after E14.5 (15). This transient population in the FL may be due to a local immediate requirement for macrophages, at least during the onset of FL hematopoiesis, to perform efficient enucleation of primitive erythrocytes passing through the FL sinusoids (100, 113). Combining historical evidences showing their direct lineage connection with the emergence of YS macrophages and recent findings showing their independence with c-Myb activity, we propose that CSF-1R$^+$ EMPs should be designated as primitive EMPs.

Origin of Fetal Monocytes

Because adult monocytes are derived from HSCs in the BM, it would be reasonable to assume that embryonic HSCs might also give rise to fetal monocytes in the developing liver. In agreement with this hypothesis, we have identified a population in the FL similar to adult MDPs that have the potential to generate fetal cMoPs and monocytes following *in vitro* culture (15). Exploiting the Flt3-Cre tomato fate-mapping model (83), we then followed the progeny of embryonic HSCs. However, the poor labeling observed between E14.5 and E17.5 in FL monocytes and macrophages contrasted with the strong labeling of FL MDPs, suggesting that HSCs had limited involvement in the generation of fetal monocytes (15). Nonetheless, the limited but significant labeling in fetal monocytes and macrophages at birth suggested an increasing derivation from fetal HSCs, assuming that fetal HSCs follow a similar Flt3-dependent differentiation pathway as adult HSCs. In parallel, gene array analysis highlighted a strong lymphoid signature within fetal MDPs (15), indicative of their derivation from the recently described YS-derived LMPs (67). Thus, LMPs may be important for the generation of a small but significant proportion of fetal monocytes prior to the expansion of mature HSCs (**Figure 4**). Further investigations using more specific fate-mapping models will be necessary to elucidate the exact contribution of LMPs as well as the hematopoietic transition between the FL and the BM.

Importantly, we observed that fetal monocytes were not tagged with the CSF-1R-Cre model that label early CSF-1R$^+$ EMPs, suggesting that fetal monopoiesis is not dependent on CSF-1R$^+$ EMPs, consistent with our previous data (9, 11) and with our YS macrophage depletion results (15, 98). Furthermore, the Runx1-iCre fate-mapping model allowed us to identify two waves of EMPs that arise sequentially before LMPs in the YS. These included an early wave, arising at E7.5 that differentiates locally into YS macrophages; and a later wave tagged at E8.5, that migrates and seeds the FL following the establishment of the blood circulation before E9.0. Early EMPs tagged at E7.5 were therefore related to those described previously by Kierdorf and Perdiguero (14, 112). The late EMPs tagged at E8.5, however, expressed c-Myb, expanded more efficiently in the FL, and differentiated *in vivo* into fetal cMoPs, constituting the major component of the fetal monocyte population as well as the fetal monocyte-derived macrophage population (**Figure 3**), which was able to maintain itself in all tissues tested (15).

The existence of two distinct EMP waves is in agreement with Bertrand et al. who reported an early wave of macrophage progenitors restricted to the YS, and a second wave that was able to reach the FL to participate in definitive hematopoiesis (42). The differential expression of c-Myb between early and late EMPs is in agreement with previous reports indicating that primitive hematopoiesis can occur in the absence of c-Myb, especially for the generation of monopotent macrophage progenitors (114), whereas EMPs from definitive hematopoiesis express and are dependent on c-Myb activity (45, 62, 115).

Notably, a previous study showed that c-Myb ablation strongly compromises definitive hematopoiesis (116). Palis et al. observed that c-Myb is expressed prior to and during the early development of definitive erythrocyte progenitors (45). Thus, late EMPs and LMPs, as well as HSCs, express c-Myb (15, 45, 61, 62), suggesting that the entire fetal monopoiesis machinery is reliant on this transcription factor. In agreement, the CD11b^{hi}F480^{lo} population, which in our hands contains fetal monocytes, was completely absent in the c-Myb-deficient embryo (12, 116). As a consequence, the contribution of c-Myb-dependent progenitors to tissue-resident macrophage populations could not be evaluated in c-Myb-deficient embryos, where c-Myb-independent YS macrophages maintain themselves as a compensatory mechanism due to the absence of c-Myb-dependent fetal monocytes that normally outcompete them. Because c-Myb expression is upregulated during the successive steps of fetal monopoiesis (15), the switch in EMP localization between the YS and the FL may indeed be orchestrated by c-Myb. As a consequence, most tissue-resident macrophages derived from fetal monocytes would therefore rely on c-Myb activity. Altogether we propose that c-Myb$^+$ EMPs giving rise to the first circulating monocytes should be designated as definitive EMPs.

Conclusion

Recent reports have drastically changed the view of the development of the MPS and shed light on the multiple layers that define fetal hematopoiesis. It is now evident that fetal monocytes form the major precursors of most adult tissue-resident macrophages, and further investigations are now necessary to clarify how they shape macrophage heterogeneity. Examining how tissues imprint specific fates in these circulating precursors will aid our understanding of the mechanisms that control the tissue-specific functions of macrophages in the steady state, and thus may uncover new therapeutic opportunities in diverse pathological settings such as metabolic diseases, fibrosis, and carcinogenesis.

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References

1. Cavaillon JM. The historical milestones in the understanding of leukocyte biology initiated by Elie Metchnikoff. *J Leukoc Biol* (2011) **90**:413–24. doi:10.1189/jlb.0211094
2. Tauber AI. Metchnikoff and the phagocytosis theory. *Nat Rev Mol Cell Biol* (2003) **4**:897–901. doi:10.1038/nrm1244
3. Yona S, Gordon S. From the reticuloendothelial to mononuclear phagocyte system – the unaccounted years. *Front Immunol* (2015) **6**:328. doi:10.3389/fimmu.2015.00328
4. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature* (2013) **496**:445–55. doi:10.1038/nature12034
5. McNelis JC, Olefsky JM. Macrophages, immunity, and metabolic disease. *Immunity* (2014) **41**:36–48. doi:10.1016/j.immuni.2014.05.010
6. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol* (2013) **13**:709–21. doi:10.1038/nri3520
7. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* (2014) **41**:49–61. doi:10.1016/j.immuni.2014.06.010
8. Prinz M, Priller J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat Rev Neurosci* (2014) **15**:300–12. doi:10.1038/nrn3722
9. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) **330**:841–5. doi:10.1126/science.1194637
10. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* (2013) **210**:1977–92. doi:10.1084/jem.20131199
11. Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med* (2012) **209**:1167–81. doi:10.1084/jem.20120340
12. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**:86–90. doi:10.1126/science.1219179
13. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**:79–91. doi:10.1016/j.immuni.2012.12.001
14. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2015) **518**:547–51. doi:10.1038/nature13989
15. Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, et al. C-myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* (2015) **42**:665–78. doi:10.1016/j.immuni.2015.03.011
16. Chow A, Brown BD, Merad M. Studying the mononuclear phagocyte system in the molecular age. *Nat Rev Immunol* (2011) **11**:788–98. doi:10.1038/nri3087
17. Van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. *J Exp Med* (1968) **128**:415–35. doi:10.1084/jem.128.3.415
18. Van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ* (1972) **46**:845–52.
19. Virolainen M. Hematopoietic origin of macrophages as studied by chromosome markers in mice. *J Exp Med* (1968) **127**:943–52. doi:10.1084/jem.127.5.943
20. Auffray C, Fogg DK, Narni-Mancinelli E, Senechal B, Trouillet C, Saederup N, et al. CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. *J Exp Med* (2009) **206**:595–606. doi:10.1084/jem.20081385
21. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* (2013) **14**:821–30. doi:10.1038/ni.2638
22. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* (2003) **19**:71–82. doi:10.1016/S1074-7613(03)00174-2
23. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) **15**:929–37. doi:10.1038/ni.2967
24. Molawi K, Wolf Y, Kandalla PK, Favret J, Hagemeyer N, Frenzel K, et al. Progressive replacement of embryo-derived cardiac macrophages with age. *J Exp Med* (2014) **211**:2151–8. doi:10.1084/jem.20140639
25. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* (2013) **39**:925–38. doi:10.1016/j.immuni.2013.10.004
26. Hashimoto K, Tarnowski WM. Some new aspects of the Langerhans cell. *Arch Dermatol* (1968) **97**:450–64. doi:10.1001/archderm.1968.01610100090015
27. Krueger GG, Daynes RA, Emam M. Biology of Langerhans cells: selective migration of Langerhans cells into allogeneic and xenogeneic grafts on nude mice. *Proc Natl Acad Sci U S A* (1983) **80**:1650–4. doi:10.1073/pnas.80.6.1650
28. Czernielewski J, Vaigot P, Prunieras M. Epidermal Langerhans cells – a cycling cell population. *J Invest Dermatol* (1985) **84**:424–6. doi:10.1111/1523-1747.ep12265523
29. Tarling JD, Lin HS, Hsu S. Self-renewal of pulmonary alveolar macrophages: evidence from radiation chimera studies. *J Leukoc Biol* (1987) **42**:443–6.
30. Naito M, Takahashi K. The role of Kupffer cells in glucan-induced granuloma formation in the liver of mice depleted of blood monocytes by administration of strontium-89. *Lab Invest* (1991) **64**:664–74.
31. Yamada M, Naito M, Takahashi K. Kupffer cell proliferation and glucan-induced granuloma formation in mice depleted of blood monocytes by strontium-89. *J Leukoc Biol* (1990) **47**:195–205.
32. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* (2007) **10**:1538–43. doi:10.1038/nn2014
33. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**:792–804. doi:10.1016/j.immuni.2013.04.004
34. Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, et al. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* (2013) **39**:599–610. doi:10.1016/j.immuni.2013.08.007
35. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, van Rooijen N, et al. Local macrophage proliferation, rather than recruitment from the

blood, is a signature of TH2 inflammation. *Science* (2011) **332**:1284–8. doi:10.1126/science.1204351
36. Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, et al. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol* (2002) **3**:1135–41. doi:10.1038/ni852
37. Romani N, Schuler G, Fritsch P. Ontogeny of Ia-positive and Thy-1-positive leukocytes of murine epidermis. *J Invest Dermatol* (1986) **86**:129–33. doi:10.1111/1523-1747.ep12284135
38. Takahashi K, Takahashi H, Naito M, Sato T, Kojima M. Ultrastructural and functional development of macrophages in the dermal tissue of rat fetuses. *Cell Tissue Res* (1983) **232**:539–52. doi:10.1007/BF00216427
39. Mizoguchi S, Takahashi K, Takeya M, Naito M, Morioka T. Development, differentiation, and proliferation of epidermal Langerhans cells in rat ontogeny studied by a novel monoclonal antibody against epidermal Langerhans cells, RED-1. *J Leukoc Biol* (1992) **52**:52–61.
40. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* (2008) **132**:631–44. doi:10.1016/j.cell.2008.01.025
41. Tavian M, Peault B. Embryonic development of the human hematopoietic system. *Int J Dev Biol* (2005) **49**:243–50. doi:10.1387/ijdb.041957mt
42. Bertrand JY, Jalil A, Klaine M, Jung S, Cumano A, Godin I. Three pathways to mature macrophages in the early mouse yolk sac. *Blood* (2005) **106**:3004–11. doi:10.1182/blood-2005-02-0461
43. Cline MJ, Moore MA. Embryonic origin of the mouse macrophage. *Blood* (1972) **39**:842–9.
44. Palis J, Chan RJ, Koniski A, Patel R, Starr M, Yoder MC. Spatial and temporal emergence of high proliferative potential hematopoietic precursors during murine embryogenesis. *Proc Natl Acad Sci U S A* (2001) **98**:4528–33. doi:10.1073/pnas.071002398
45. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. *Development* (1999) **126**:5073–84.
46. Tober J, Koniski A, McGrath KE, Vemishetti R, Emerson R, de Mesy-Bentley KK, et al. The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. *Blood* (2007) **109**:1433–41. doi:10.1182/blood-2006-06-031898
47. Frame JM, McGrath KE, Palis J. Erythro-myeloid progenitors: "definitive" hematopoiesis in the conceptus prior to the emergence of hematopoietic stem cells. *Blood Cells Mol Dis* (2013) **51**:220–5. doi:10.1016/j.bcmd.2013.09.006
48. Gulliver G. Observations on the sizes and shapes of red corpuscles of the blood of vertebrates, with drawings of them to a uniform scale, and extended and revised tables of measurements. *Proc Zool Soc Lond* (1875):474–95.
49. Palis J. Primitive and definitive erythropoiesis in mammals. *Front Physiol* (2014) **5**:3. doi:10.3389/fphys.2014.00003
50. Naito M, Yamamura F, Nishikawa S, Takahashi K. Development, differentiation, and maturation of fetal mouse yolk sac macrophages in cultures. *J Leukoc Biol* (1989) **46**:1–10.
51. Takahashi K, Naito M. Development, differentiation, and proliferation of macrophages in the rat yolk sac. *Tissue Cell* (1993) **25**:351–62. doi:10.1016/0040-8166(93)90077-X
52. Takahashi K, Yamamura F, Naito M. Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: a light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. *J Leukoc Biol* (1989) **45**:87–96.
53. Faust N, Huber MC, Sippel AE, Bonifer C. Different macrophage populations develop from embryonic/fetal and adult hematopoietic tissues. *Exp Hematol* (1997) **25**:432–44.
54. Morioka Y, Naito M, Sato T, Takahashi K. Immunophenotypic and ultrastructural heterogeneity of macrophage differentiation in bone marrow and fetal hematopoiesis of mouse in vitro and in vivo. *J Leukoc Biol* (1994) **55**:642–51.
55. Lin Y, Yoder MC, Yoshimoto M. Lymphoid progenitor emergence in the murine embryo and yolk sac precedes stem cell detection. *Stem Cells Dev* (2014) **23**:1168–77. doi:10.1089/scd.2013.0536
56. McGrath KE, Koniski AD, Malik J, Palis J. Circulation is established in a stepwise pattern in the mammalian embryo. *Blood* (2003) **101**:1669–76. doi:10.1182/blood-2002-08-2531
57. Bertrand JY, Kim AD, Violette EP, Stachura DL, Cisson JL, Traver D. Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo. *Development* (2007) **134**:4147–56. doi:10.1242/dev.012385
58. Dzierzak E, Speck NA. Of lineage and legacy: the development of mammalian hematopoietic stem cells. *Nat Immunol* (2008) **9**:129–36. doi:10.1038/ni1560
59. Ferkowicz MJ, Starr M, Xie X, Li W, Johnson SA, Shelley WC, et al. CD41 expression defines the onset of primitive and definitive hematopoiesis in the murine embryo. *Development* (2003) **130**:4393–403. doi:10.1242/dev.00632
60. Mikkola HK, Orkin SH. The journey of developing hematopoietic stem cells. *Development* (2006) **133**:3733–44. doi:10.1242/dev.02568
61. Godin I, Dieterlen-Lievre F, Cumano A. Emergence of multipotent hemopoietic cells in the yolk sac and paraaortic splanchnopleura in mouse embryos, beginning at 8.5 days postcoitus. *Proc Natl Acad Sci U S A* (1995) **92**:773–7. doi:10.1073/pnas.92.23.10815b
62. Yoder MC, Hiatt K, Dutt P, Mukherjee P, Bodine DM, Orlic D. Characterization of definitive lymphohematopoietic stem cells in the day 9 murine yolk sac. *Immunity* (1997) **7**:335–44. doi:10.1016/S1074-7613(00)80355-6
63. Lacaud G, Carlsson L, Keller G. Identification of a fetal hematopoietic precursor with B cell, T cell, and macrophage potential. *Immunity* (1998) **9**:827–38. doi:10.1016/S1074-7613(00)80648-2
64. Adolfsson J, Mansson R, Buza-Vidas N, Hultquist A, Liuba K, Jensen CT, et al. Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* (2005) **121**:295–306. doi:10.1016/j.cell.2005.02.013
65. Yoshimoto M, Montecino-Rodriguez E, Ferkowicz MJ, Porayette P, Shelley WC, Conway SJ, et al. Embryonic day 9 yolk sac and intra-embryonic hemogenic endothelium independently generate a B-1 and marginal zone progenitor lacking B-2 potential. *Proc Natl Acad Sci U S A* (2011) **108**:1468–73. doi:10.1073/pnas.1015841108
66. Yoshimoto M, Porayette P, Glosson NL, Conway SJ, Carlesso N, Cardoso AA, et al. Autonomous murine T-cell progenitor production in the extra-embryonic yolk sac before HSC emergence. *Blood* (2012) **119**:5706–14. doi:10.1182/blood-2011-12-397489
67. Boiers C, Carrelha J, Lutteropp M, Luc S, Green JC, Azzoni E, et al. Lymphomyeloid contribution of an immune-restricted progenitor emerging prior to definitive hematopoietic stem cells. *Cell Stem Cell* (2013) **13**:535–48. doi:10.1016/j.stem.2013.08.012
68. Kieusseian A, Brunet de la Grange P, Burlen-Defranoux O, Godin I, Cumano A. Immature hematopoietic stem cells undergo maturation in the fetal liver. *Development* (2012) **139**:3521–30. doi:10.1242/dev.079210
69. Cumano A, Dieterlen-Lievre F, Godin I. Lymphoid potential, probed before circulation in mouse, is restricted to caudal intraembryonic splanchnopleura. *Cell* (1996) **86**:907–16. doi:10.1016/S0092-8674(00)80166-X
70. McGrath KE, Frame JM, Fegan KH, Bowen JR, Conway SJ, Catherman SC, et al. Distinct sources of hematopoietic progenitors emerge before HSCs and provide functional blood cells in the mammalian embryo. *Cell Rep* (2015) **11**:1892–904. doi:10.1016/j.celrep.2015.05.036
71. Muller AM, Medvinsky A, Strouboulis J, Grosveld F, Dzierzak E. Development of hematopoietic stem cell activity in the mouse embryo. *Immunity* (1994) **1**:291–301. doi:10.1016/1074-7613(94)90081-7
72. Tavian M, Robin C, Coulombel L, Peault B. The human embryo, but not its yolk sac, generates lympho-myeloid stem cells: mapping multipotent hematopoietic cell fate in intraembryonic mesoderm. *Immunity* (2001) **15**:487–95. doi:10.1016/S1074-7613(01)00193-5
73. Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK. The placenta is a niche for hematopoietic stem cells. *Dev Cell* (2005) **8**:365–75. doi:10.1016/j.devcel.2004.12.016
74. Mikkola HK, Gekas C, Orkin SH, Dieterlen-Lievre F. Placenta as a site for hematopoietic stem cell development. *Exp Hematol* (2005) **33**:1048–54. doi:10.1016/j.exphem.2005.06.011
75. de Bruijn MF, Speck NA, Peeters MC, Dzierzak E. Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. *EMBO J* (2000) **19**:2465–74. doi:10.1093/emboj/19.11.2465
76. Kumaravelu P, Hook L, Morrison AM, Ure J, Zhao S, Zuyev S, et al. Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver. *Development* (2002) **129**:4891–9.
77. Golub R, Cumano A. Embryonic hematopoiesis. *Blood Cells Mol Dis* (2013) **51**:226–31. doi:10.1016/j.bcmd.2013.08.004

78. Medvinsky A, Rybtsov S, Taoudi S. Embryonic origin of the adult hematopoietic system: advances and questions. *Development* (2011) **138**:1017–31. doi:10.1242/dev.040998
79. Swiers G, Rode C, Azzoni E, de Bruijn MF. A short history of hemogenic endothelium. *Blood Cells Mol Dis* (2013) **51**:206–12. doi:10.1016/j.bcmd.2013.09.005
80. Christensen JL, Wright DE, Wagers AJ, Weissman IL. Circulation and chemotaxis of fetal hematopoietic stem cells. *PLoS Biol* (2004) **2**:E75. doi:10.1371/journal.pbio.0020075
81. Kawamoto H, Katsura Y. A new paradigm for hematopoietic cell lineages: revision of the classical concept of the myeloid-lymphoid dichotomy. *Trends Immunol* (2009) **30**:193–200. doi:10.1016/j.it.2009.03.001
82. Oguro H, Ding L, Morrison SJ. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. *Cell Stem Cell* (2013) **13**:102–16. doi:10.1016/j.stem.2013.05.014
83. Boyer SW, Schroeder AV, Smith-Berdan S, Forsberg EC. All hematopoietic cells develop from hematopoietic stem cells through Flk2/Flt3-positive progenitor cells. *Cell Stem Cell* (2011) **9**:64–73. doi:10.1016/j.stem.2011.04.021
84. Coskun S, Chao H, Vasavada H, Heydari K, Gonzales N, Zhou X, et al. Development of the fetal bone marrow niche and regulation of HSC quiescence and homing ability by emerging osteolineage cells. *Cell Rep* (2014) **9**:581–90. doi:10.1016/j.celrep.2014.09.013
85. Chen MJ, Li Y, De Obaldia ME, Yang Q, Yzaguirre AD, Yamada-Inagawa T, et al. Erythroid/myeloid progenitors and hematopoietic stem cells originate from distinct populations of endothelial cells. *Cell Stem Cell* (2011) **9**:541–52. doi:10.1016/j.stem.2011.10.003
86. Samokhvalov IM, Samokhvalova NI, Nishikawa S. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* (2007) **446**:1056–61. doi:10.1038/nature05725
87. Sheng J, Ruedl C, Karjalainen K. Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. *Immunity* (2015) **43**:382–93. doi:10.1016/j.immuni.2015.07.016
88. Chen MJ, Yokomizo T, Zeigler BM, Dzierzak E, Speck NA. Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature* (2009) **457**:887–91. doi:10.1038/nature07619
89. Cumano A, Godin I. Ontogeny of the hematopoietic system. *Annu Rev Immunol* (2007) **25**:745–85. doi:10.1146/annurev.immunol.25.022106.141538
90. Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature* (2010) **464**:116–20. doi:10.1038/nature08764
91. Busch K, Klapproth K, Barile M, Flossdorf M, Holland-Letz T, Schlenner SM, et al. Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. *Nature* (2015) **518**:542–6. doi:10.1038/nature14242
92. Naito M, Takahashi K, Nishikawa S. Development, differentiation, and maturation of macrophages in the fetal mouse liver. *J Leukoc Biol* (1990) **48**:27–37.
93. Lichanska AM, Hume DA. Origins and functions of phagocytes in the embryo. *Exp Hematol* (2000) **28**:601–11. doi:10.1016/S0301-472X(00)00157-0
94. Naito M, Umeda S, Yamamoto T, Moriyama H, Umezu H, Hasegawa G, et al. Development, differentiation, and phenotypic heterogeneity of murine tissue macrophages. *J Leukoc Biol* (1996) **59**:133–8.
95. Sorokin SP, McNelly NA, Blunt DG, Hoyt RF Jr. Macrophage development: III. Transformation of pulmonary macrophages from precursors in fetal lungs and their later maturation in organ culture. *Anat Rec* (1992) **232**:551–71. doi:10.1002/ar.1092320411
96. Hopkinson-Woolley J, Hughes D, Gordon S, Martin P. Macrophage recruitment during limb development and wound healing in the embryonic and foetal mouse. *J Cell Sci* (1994) **107**(Pt 5):1159–67.
97. Wood W, Turmaine M, Weber R, Camp V, Maki RA, McKercher SR, et al. Mesenchymal cells engulf and clear apoptotic footplate cells in macrophage-less PU.1 null mouse embryos. *Development* (2000) **127**:5245–52.
98. Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, et al. Microglia modulate wiring of the embryonic forebrain. *Cell Rep* (2014) **8**:1271–9. doi:10.1016/j.celrep.2014.07.042
99. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* (2014) **40**:91–104. doi:10.1016/j.immuni.2013.11.019
100. Naito M, Hasegawa G, Takahashi K. Development, differentiation, and maturation of Kupffer cells. *Microsc Res Tech* (1997) **39**:350–64. doi:10.1002/(SICI)1097-0029(19971115)39:4<350::AID-JEMT5>3.3.CO;2-V
101. Schneider C, Nobs SP, Kurrer M, Rehrauer H, Thiele C, Kopf M. Induction of the nuclear receptor PPAR-gamma by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages. *Nat Immunol* (2014) **15**:1026–37. doi:10.1038/ni.3005
102. Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* (2010) **468**:562–6. doi:10.1038/nature09513
103. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat Med* (2013) **19**:1166–72. doi:10.1038/nm.3258
104. van de Pavert SA, Mebius RE. New insights into the development of lymphoid tissues. *Nat Rev Immunol* (2010) **10**:664–74. doi:10.1038/nri2832
105. Randall TD, Carragher DM, Rangel-Moreno J. Development of secondary lymphoid organs. *Annu Rev Immunol* (2008) **26**:627–50. doi:10.1146/annurev.immunol.26.021607.090257
106. Kubota Y, Takubo K, Shimizu T, Ohno H, Kishi K, Shibuya M, et al. M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. *J Exp Med* (2009) **206**:1089–102. doi:10.1084/jem.20081605
107. Gordon EJ, Rao S, Pollard JW, Nutt SL, Lang RA, Harvey NL. Macrophages define dermal lymphatic vessel calibre during development by regulating lymphatic endothelial cell proliferation. *Development* (2010) **137**:3899–910. doi:10.1242/dev.050021
108. Gray EE, Cyster JG. Lymph node macrophages. *J Innate Immun* (2012) **4**:424–36. doi:10.1159/000337007
109. Haldar M, Kohyama M, So AY, Kc W, Wu X, Briseno CG, et al. Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. *Cell* (2014) **156**:1223–34. doi:10.1016/j.cell.2014.01.069
110. A-Gonzalez N, Guillen JA, Gallardo G, Diaz M, de la Rosa JV, Hernandez IH, et al. The nuclear receptor LXRalpha controls the functional specialization of splenic macrophages. *Nat Immunol* (2013) **14**:831–9. doi:10.1038/ni.2622
111. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
112. Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat Neurosci* (2013) **16**:273–80. doi:10.1038/nn.3318
113. McGrath KE, Kingsley PD, Koniski AD, Porter RL, Bushnell TP, Palis J. Enucleation of primitive erythroid cells generates a transient population of "pyrenocytes" in the mammalian fetus. *Blood* (2008) **111**:2409–17. doi:10.1182/blood-2007-08-107581
114. Clarke D, Vegiopoulos A, Crawford A, Mucenski M, Bonifer C, Frampton J. In vitro differentiation of c-myb(-/-) ES cells reveals that the colony forming capacity of unilineage macrophage precursors and myeloid progenitor commitment are c-Myb independent. *Oncogene* (2000) **19**:3343–51. doi:10.1038/sj.onc.1203661
115. Sumner R, Crawford A, Mucenski M, Frampton J. Initiation of adult myelopoiesis can occur in the absence of c-Myb whereas subsequent development is strictly dependent on the transcription factor. *Oncogene* (2000) **19**:3335–42. doi:10.1038/sj.onc.1203660
116. Mucenski ML, McLain K, Kier AB, Swerdlow SH, Schreiner CM, Miller TA, et al. A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. *Cell* (1991) **65**:677–89. doi:10.1016/0092-8674(91)90099-K

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REVIEW
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Transcriptional regulation of mononuclear phagocyte development

Roxane Tussiwand[1] and Emmanuel L. Gautier[2]*

[1] Department of Biomedicine, University of Basel, Basel, Switzerland, [2] INSERM UMR_S 1166, Sorbonne Universités, UPMC Univ Paris 06, Pitié-Salpêtrière Hospital, Paris, France

Mononuclear phagocytes (MP) are a quite unique subset of hematopoietic cells, which comprise dendritic cells (DC), monocytes as well as monocyte-derived and tissue-resident macrophages. These cells are extremely diverse with regard to their origin, their phenotype as well as their function. Developmentally, DC and monocytes are constantly replenished from a bone marrow hematopoietic progenitor. The ontogeny of macrophages is more complex and is temporally linked and specified by the organ where they reside, occurring early during embryonic or perinatal life. The functional heterogeneity of MPs is certainly a consequence of the tissue of residence and also reflects the diverse ontogeny of the subsets. In this review, we will highlight the developmental pathways of murine MP, with a particular emphasis on the transcriptional factors that regulate their development and function. Finally, we will discuss and point out open questions in the field.

Keywords: transcription factors, development, dendritic cells, macrophages, immunity

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****Correspondence:***
Roxane Tussiwand
r.tussiwand@unibas.ch

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INTRODUCTION

The mononuclear-phagocyte system (MPS), which comprises dendritic cells (DCs), macrophages, and monocytes, is a heterogeneous group of myeloid cells. The complexity of the MPS is equally reflected by the plasticity in function and phenotype that characterizes each subset depending on their location and activation state. Specialized subsets of mononuclear phagocytes (MP) reside in defined anatomical locations, are critical for the homeostatic maintenance of tissues, and provide the link between innate and adaptive immune responses during infections. The ability of MP to maintain or to induce the correct tolerogenic or inflammatory milieu also resides in their complex subset specialization. Such subset heterogeneity is obtained through lineage diversification and specification, which is controlled by defined transcriptional networks and programs. Understanding the MP biology means to define their transcriptional signature, which is required during lineage commitment, and which characterizes each subset's features. This review will focus on the transcriptional regulation of the MPS; in particular, what determines lineage commitment and functional identity; we will emphasize recent advances in the field of single-cell analysis and highlight unresolved questions in the field.

THE MONONUCLEAR-PHAGOCYTE SYSTEM NETWORK

As summarized in **Table 1**, the MPS is a rather heterogeneous group of myeloid cells, which includes DC, monocytes, and macrophages (1). DCs are mostly short lived and characterized by a half-life that varies between few days up to few weeks (2). This subset of MPs is equipped with pattern recognition receptors (PRR) and is specialized in antigen capture and presentation to T cells (3). At least three different DC

subsets have been identified: plasmacytoid DCs (pDCs), and two common or conventional DC (cDC) subsets; cDC1, which express CD24, and CD8α in lymphoid tissues, or CD103 in peripheral organs; and cDC2, which express CD4, CD11b, and CD172 (1). This latter subset of cDCs is heterogeneous and seems to comprise also monocyte-derived DCs and activated macrophages, which have acquired a DC phenotype and most likely function (4).

Steady-state monocytes are short-lived MPs. They are subdivided into two major subsets: patrolling and inflammatory monocytes, which are characterized by low and high expression of Ly-6C, respectively (5). Inflammatory monocytes are recruited and extravasate into infected tissues. They play a role in maintaining the correct inflammatory milieu, are important in the resolution of inflammation and in certain tissues monocytes will replenish the pool of resident macrophages (5–7). The role of patrolling monocytes is less clear but they are certainly involved in the homeostasis of the endothelium (8, 9).

The last subset of MP comprises the mostly long-lived tissue-resident macrophages (10). This subset is present in every developing as well as mature tissue, which is highly heterogeneous in terms of phenotype and function, reflecting the physiological needs of the organ of origin (11). Macrophages are thought to be required for the correct development and maintenance of tissues. This topological-related feature is possibly the reason for their extreme heterogeneity and their tissue specialization (12).

Collectively, MPs are highly plastic myeloid cells, which can perform very diverse functions. **Table 1** summarizes the mostly used surface markers in mice and the function attributed to the different MP subsets.

TRANSCRIPTIONAL REGULATION OF DENDRITIC CELLS DEVELOPMENT

As shown in **Figure 1**, lineage development of hematopoietic progenitor cells along DC lineage occurs through an orchestrated expression pattern of transcription factors (TF), yet the precise molecular mechanisms of lineage restriction and determination remains largely unexplained (2, 13–17). The analysis of gene-targeted mice has revealed the functional importance of a few critical TFs in DC development, with some of them affecting all DCs and some affecting specific subsets (18). DC progenitors are present within the fms-related tyrosine kinase 3 (Flt3)-expressing bone marrow fraction and sustained Flt3 signaling can be considered as instructive for DC development (19–22). Consistently, Flt3-ligand (Flt3L) supports the *in vitro* differentiation of progenitor cells into both pDCs and cDCs (23, 24). Genetic deletion of Flt3L, its receptor, or treatment of mice with Flt3 inhibitors leads to a 10-fold reduction of lymphoid-organ pDCs and cDCs (25, 26). Moreover, Flt3L injection or overexpression of Flt3L results in the expansion of both pDCs and cDCs in all lymphoid and non-lymphoid organs (27, 28). Engagement of Flt3 by Flt3L induces Stat3 phosphorylation and activation, identifying Stat3 as the critical checkpoint of Flt3-induced DC development and proliferation (29, 30). Mirroring Flt3 deficiency, Stat3-deficient mice have severely reduced DC progenitors and mature cells (29). Similarly, deletion of the transcriptional repressor growth factor independent 1 (Gfi1) results in impaired DC development (31). Gfi1-deficient mice show reduced Stat3 phosphorylation and nuclear translocation, with increased expression levels of the Stat3 negative regulators SOCS3 and PIAS3 suggesting that Gfi1 is downstream of Stat3 signaling in the Flt3-Flt3L-induced DC developmental pathway (31). However, the role of Gfi1 is more complex since mice deficient for this repressor show multiple hematopoietic impairments (32, 33). The defects related to Gfi1 deficiency can partially be related to dysregulation of Id2 expression (34–36). However, further studies using subset-specific deletion models will be instrumental to precisely dissect specific transcriptional requirements within the MP lineage. Similarly, despite the experimental evidence of DC expansion following sustained Flt3 signaling, the instructive mechanism promoting DC development is still unclear, given the broad expression of Flt3 on all short-term uncommitted hematopoietic progenitors (ST-HSC) (37, 38). A long non-coding RNA (lncRNA), named lnc-DC, was recently suggested to be the missing key element regulating Stat3 activity exclusively in DCs (39). lnc-DC RNA is expressed by mature DCs and by monocyte-derived DCs and seems to directly interact with Stat3 preventing its de-phosphorylation by SHP1. Furthermore, knockdown experiments of lnc-DC *in vitro* showed impaired DC development from mouse BM progenitors. The conservation of this lnc-DC in terms of function and of its consensus elements at the promoter region across species supports the hypothesis of a new level of regulation present in DC development. However, in mice the transcript seems translated into a highly expressed protein in adipose tissue (40). Further studies are therefore needed to understand potential species-specificities as well as its requirement *in vivo* under steady-state conditions.

Proceeding along the DC developmental pathway, three major branches of mature DCs are identified: pDCs, $CD24^+$ cDC1, and $CD11b^+$ cDC2 (3, 16). pDCs and cDC1 both express and depend on the transcription factor interferon regulatory factor 8 (Irf8), while cDC2 express and are partially dependent on Irf4 (1, 18, 41–44). Despite major advances in our understanding of the transcriptional requirement during DC development, we are still unable to draw a clear developmental map (**Figure 2**) (13, 18). This may reflect subset heterogeneity as well as the plasticity, which characterizes DCs. Also, the expression of the different TFs

TABLE 1 | Summarized are the three major murine MPs: dendritic cells, monocytes, and macrophages.

MPs	Subset	Surface MK	Functions
Dendritic cells	pDCs	SiglecH, Bst2	Production of type 1 IFN (antiviral response)
	cDC1	XCR1, CD103/ CD8, Clec9a	Th1 and CTL immunity, cross-presentation, IL-12 production
	cDC2	CD11b, Sirp-α	Th2 and Th17 immunity, production of IL-23 and IL-6
Monocytes	Ly6C high inflammatory	Ly6C hi CCR2 hi	Differentiate into DCs and tissue macrophages during inflammation
	Ly6C low patrolling	Ly6C low CCR2 low Cx3Cr1	Endothelial integrity
Macrophages	Tissue specific	F4/80, MerTK, CD64 CD11b	Tissue specific

Each MP subset can be further subdivided into different subsets based on surface marker expression and function and as indicated.

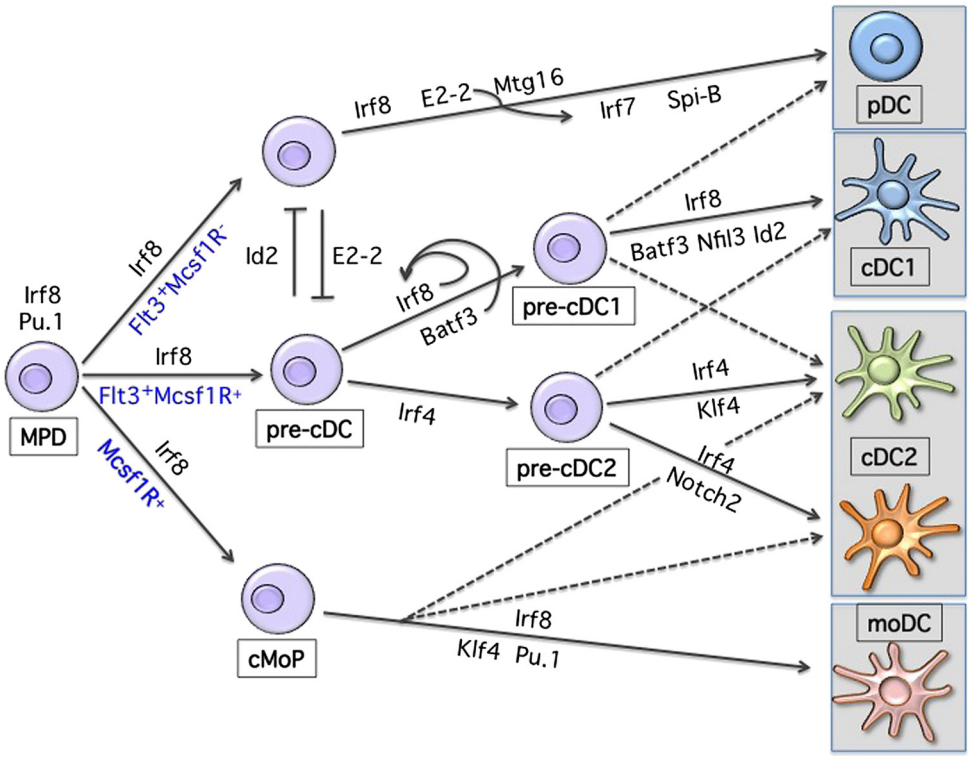

FIGURE 1 | Transcriptional development of dendritic cells. Shown are the major transcription factors known to be involved in DC lineage commitment. Development occurs from a Flt3-, Irf8-expressing hematopoietic progenitor. Progressive acquisition of one or more TFs will result in differentiation toward a specific MP subset. Loss or reduction of one or more TFs can, to some extent, redirect commitment to another lineage.

is not unique and can change during differentiation and activation further complicating the picture.

During early stages of DC development, a progenitor that expresses high levels of Irf8 and shows developmental potential toward all DCs can be identified (42). It is likely that the first branching choice will determine whether pDCs or cDCs commitment occurs. The balance of the E-protein transcription factor 4 (Tcf4), also known as E2-2, and the E-protein inhibitor of DNA binding 2 (Id2) seems to determine lineage development toward pDCs or cDCs, respectively (45–50). Constitutive or inducible deletion of E2-2 in CD11c-expressing cells blocks the development of pDCs but not cDCs, while overexpression of Id2 inhibits pDC development (47, 48). E2-2 is required not only during development but also for lineage maintenance of pDCs (47, 51). Several targets of E2-2 have been identified such as SpiB, Irf8, and Irf7, and all contribute to pDCs lineage specification (47, 51). Despite the requirement for E2-2 during pDCs commitment, how Id2 and E2-2 are conversely induced and regulated is still an open question. Recently, the eight-twenty-one (ETO) protein 2 or Mtg16 (also referred as core-binding factor, runt domain, alpha subunit 2, translocated to 3 Cbfa2t3) was suggested to target and repress Id2 together with E2-2 and inhibit Irf8-expressing cDC1 development, while favoring pDC commitment (52). Consistently, Id2 and Mtg16 double-deficient mice show restored pDC potential (52). However, Mtg16 seems to act together with E2-2 leaving the question on how lineage determination toward E2-2- or Id2-expressing progenitors occurs, still open. On the other side, one other candidate, which could be involved in reinforcing lineage fate toward Irf8-expressing cDC1 at the expenses of pDCs could be the leucine zipper transcription factor E4BP4, also referred as Nfil3 (53). Mice deficient for this TF show increased pDC and reduced cDC1 development (53). The mechanism of action remains to be elucidated since Id2 expression does not appear to be perturbed and only the basic leucine zipper transcription factor ATF-like 3 (Batf3) expression was shown to be reduced (53). Phenotypically, a bias toward pDC development has been observed within the macrophage-colony-stimulating factor receptor (M-CSFR) negative progenitors, whereas cDCs precursors are enriched within the M-CSFR expressing BM fraction (54, 55). These results may suggest that under sustained M-CSF stimulation uncommitted progenitors may lose the potential toward pDCs. Alternatively, as recently suggested, the absence of GM-CSF signaling, which induces STAT5 phosphorylation, could be the permissive condition to promote pDC development (56). Accumulation and/or withdrawal of specific cytokines during proliferation and differentiation as well as regulation of TF levels through division of progenitor cells could partially explain how BM niches influence development and lineage commitment (57, 58).

Proceeding along DC development, a common cDC progenitor able to differentiate *in vivo* into both $CD24^+$ cDC1 and $CD11b^+$ cDC2 was identified (55, 59–61). And recently, lineage-tracing studies allowed further dissection of cDC commitment and resulted fundamental to establish the transcriptional

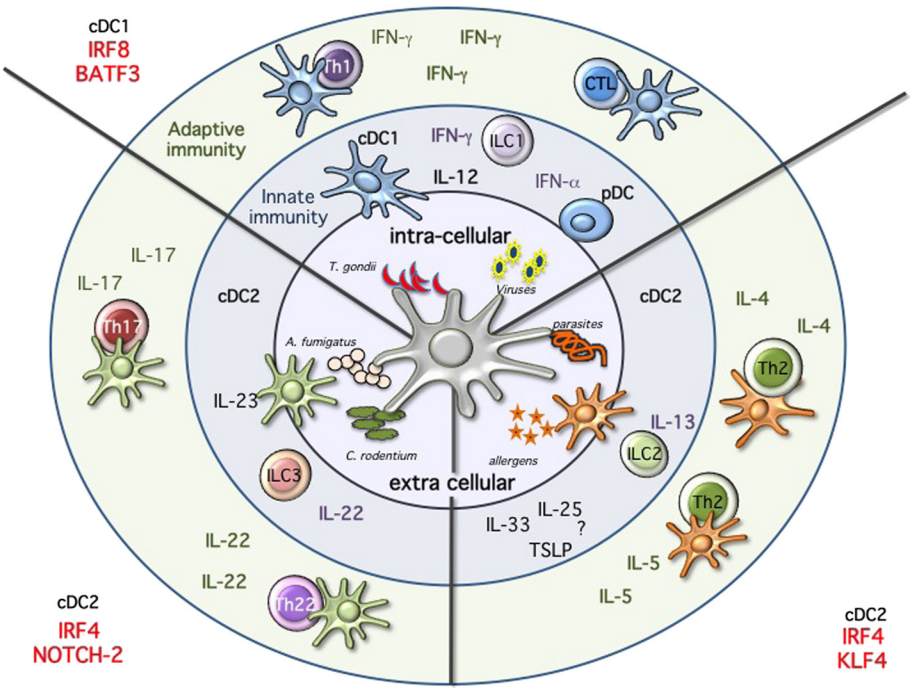

FIGURE 2 | Immune modules dendritic cells will sense the environment and start the immune response by producing cytokines, activating innate immune cells, and priming T cells. Intracellular pathogens, such as *Toxoplasma gondii*, or viral infections will activate cDC1 and pDCs. These subsets are specialized in the production of IL-12 and type 1 IFNs, respectively. High amounts of IL-12 will activate innate lymphoid cells 1 (ILC1) to produce IFN-g and ultimately leading to the priming of Th1 immunity and sustain IFN-g secretion. Following viral infections, pDCs will produce high amounts of type 1 IFNs, while cDC1 will prime CTL response through cross-presentation of infected cells. Immunity against fungi and extracellular pathogens is mostly mediated by Irf4/Notch2 cDC2, which produce high amounts of IL-23, leading to the activation of ILC3 and IL-22 production as well as priming of Th17 and Th22 T cells. Th2 immunity during allergic reactions and following parasitic infections requires KLf4-dependent cDC2. In this case, the mechanism seems to be more complex and may require ILC2, but ultimately results in the activation of Th2 cells and the production of IL-4 and IL-5.

requirements during development of clonogenic cDC progenitors (62, 63). The expression pattern of the zinc finger and BTB domain containing 46 transcription factor Zbtb46 (also called Btbd4) can be considered as cDC-lineage specific within hematopoietic cells (64–66). This TF is not present on pDCs and is induced on monocyte-derived DCs, supporting on the one hand early divergence of pDCs during DC commitment, and on the other hand suggesting a developmental convergence between cDCs and monocyte-derived DCs (4, 66). Similarly, lineage-tracing experiments were performed using mice expressing Cre recombinase under the control of Clec9a also referred as Dngr-1 (67). Although labeling is not absolute on all cDCs subsets, it seems to be restricted to pre-cDC progeny, without marking inflammatory-derived DCs (67). The use of these reporter mouse models will help us better characterize the ontogeny of specific cDCs subsets also depending on the tissue of origin and whether under steady-state or inflammatory conditions.

The CD24$^+$ cDC1 branch of cDCs depends on the transcription factors Irf8, Id2, Nfil3, and Batf3 (68). The generation of mice deficient for Batf3 has revealed the common origin and the lineage identity of Irf8-expressing cDC1 cells, also referred as CD8a$^+$ or CD103$^+$ across all lymphoid and peripheral organs (69, 70). However, while only Irf8 was shown to be necessary for commitment, Id2, Nfil3, and Batf3 are dispensable under certain conditions (71, 72). Lineage choice seems influenced by high and sustained levels of Irf8 during cDC1 commitment. Binding of Batf3 and Irf8 to an AP1-IRF composite element (AICE) within the Irf8 super-enhancer in CD24- or Zbtb46-gfp-expressing immediate progenitors leads to sustained Irf8 expression and cDC1 development (62). In the absence of Batf3, reduced Irf8 levels, redirect commitment of a CD24-expressing cDC1 progenitor toward the Irf4-expressing cDC2 lineage (62).

Despite the recent advances, how the branching of cDC1 and cDC2 occurs is still an open question. The recent identification of a committed cDC2 progenitor might help to identify the key factors involved in this process: we still need to understand how expression of Irf4 progressively replaces Irf8, and how those two TF determine the identity of these subsets. Furthermore, the cDC2 lineage, as already mentioned, is highly heterogeneous and possibly contains multiple subsets (1, 2, 11, 16, 18). Mature CD11b-expressing cDC2 express high levels of Irf4, suggesting an important role for this TF within this lineage. And indeed, absence of Irf4 impairs the development as well as the function of cDC2 (42, 44, 73–77). In mice lacking IRF4 in CD11c-expressing cells, cDC2 numbers are reduced in lung and small intestinal DCs, while no difference is reported for skin (44, 74). However, reduction in lung and lamina propria cDCs is only observed upon deletion of Irf4 in early progenitors (44; 74). Despite, normal numbers of skin DCs in Irf4-deficient animals, migration to draining lymph nodes is impaired as a consequence of defective induction of CCR7 (78). Furthermore,

reduced up-regulation of MHC-II and co-stimulatory molecules is also associated with Irf4 deficiency (75, 77, 78). Collectively, Irf4 shows a broad action across different tissues and potentially subsets, and further studies are required to be able to understand the specific requirement of this TF during development.

Other TFs reported to display a reduction of cDC2 are RelB, Notch2, RbpJ, and the Kruppel-like Factor 4 (Klf4) (79–85). Notch2 is required for terminal differentiation of endothelial cell-selective adhesion molecule (ESAM)-expressing splenic cDC (81, 83). Similar to Notch2 deficiency, mice compromised in Runx3 (86, 87) and in the alternative NF-kB pathway show a reduction in the development of ESAM$^+$ cDCs (80, 88). However, a survival disadvantage in competitive settings appears to be present in mice with compromised NF-kB signaling, suggesting caution in proposing the requirement for NF-kB during DCs development (80, 88). Klf4 deficiency results in impaired development of the so-called "double negative" DCs in skin draining LN and a partial reduction of Sirp-α but not splenic ESAM-expressing cDC2 across all the organs (84). In these mice, cDC progenitors are impaired in their ability to down-regulate Irf8 and up-regulate Irf4. However, the *in vitro* differentiation potential of Irf4-expressing cDCs as well as expression of Irf4 on peripheral cDCs is not compromised. This can be explained by the existence of at least two cDC2 subsets, where only the Klf4/Irf4-dependent one is developmentally impaired. Alternatively, a different maturation/activation state, which requires Klf4, may exist within the Irf4-expressing cDC2 subset.

Collectively, a partial reduction associated with the lack of one or the other TF confirms the developmental, and supports the subset-specific heterogeneity observed in single-cell sequencing experiments for the Irf4 and CD11b-expressing cDC2 cells (89–92). The transcriptional diversity, which characterizes these cDC2 cells, results and reflects a functional heterogeneity (**Figure 2**). Notch2 cDC2 are required for anti bacterial Th17/IL-22 immunity, while Klf4 deficiency results in impaired Th2 immunity (83, 84, 93). Expression of Irf4 in cDCs is necessary for both Th17 and Th2 responses further highlighting the complexity of this TF in DC biology (44, 73, 74, 77). Understanding whether the absence of a subset or a functional defect caused by a transcriptional deficiency on the remaining subset could account for the observed phenotypes will require subset-specific deletion. Furthermore, we also need to explore more in detail the influence of tissues on the different subsets. Are tissue-specific cues driving the expression of a transcriptional signature in a similar way as recently revealed for macrophages? (12) Are the differences reflecting a developmental or a functional heterogeneity? Is a developmental convergence between cDCs and monocyte-derived DCs creating the confusion within this branch of cDCs. We need a better characterization of the different subsets, which fall under the broad umbrella of CD11b or Irf4-expressing cDC2 and some progress has certainly been made with the introduction of new reporter mice as previously discussed as well as the recently identified committed progenitor. Teasing this heterogeneous pool of Irf4-expressing cDC2 apart is currently an active field of investigation (90, 91, 94). And new technologies will be instrumental to improve our comprehension of the molecular clues, which regulate lineage commitment. A recent report analyzed stage and subset-specific expression of mi-RNAs during DC development and miR-142 was identified as a key regulator of cDC2 differentiation, further adding additional complexity to our current understanding of DC development (91).

Better genetic models are needed and will possibly be soon developed as a result of the recently published single-cell analysis (16, 89). Identifying TFs or surface markers, which would compromise or trace the development of one lineage independently of the anatomical localization, as previously done in Batf3$^{-/-}$ mice for Irf8-dependent cDC1 would be of great advantage (69, 70).

TRANSCRIPTIONAL REGULATION OF MONOCYTE DEVELOPMENT

The molecular regulation, which defines monocyte differentiation and lineage commitment, is poorly understood (95). Most of the identified TFs, that result in impaired monocyte development, also show an effect on other hematopoietic lineages. The transcription factors Irf8, Sfpi1 (PU.1), Egr-1, Stat3, Gfi1, Gata2, Gbx2, Nur77, retinoic acid receptors, C/EBPα and C/EBPβ, Klf4, and c-Maf as well as members of the NF-κB family members are all involved in monocyte differentiation, however their function is often redundant, certainly not limited to monocytes and in some cases mediating proliferative and/or survival rather than instructive cues (96). Most of the TFs involved in monocyte differentiation are shared within the myelo-monocytic branch. Some of them were already mentioned as important during DC development; others are involved in macrophage and or granulocyte commitment; we are therefore aware that we can only provide here a simplified transcriptional path, which leads to monocyte development and that more efforts are required to better understand.

Expression of the ETS family transcription factor Sfpi1 or PU.1 at early stages is suggested to antagonize on the one hand key regulators of other developmental pathways, such as GATA-1 for erythroid lineage, and on the other hand activate myeloid-specific factors such as Irf8, Klf4, and Erg1 (95). A critical step in monocyte differentiation is the induction of Csf1R expression at the cell surface. This seems to be regulated by Klf4 and Irf8, however both factors are also involved in cDC development, as previously discussed, (85). Furthermore, Csf1R is also needed for macrophage development.

The identification of a committed progenitor with monocyte-restricted potential called cMoP confirmed high expression levels of the above-mentioned TF (97). However, none of those is unique to monocyte differentiation and potentially complex genetic models will be required to unravel the transcriptional map required for monocyte lineage specification.

ORIGIN OF TISSUE-RESIDENT MACROPHAGES

As discussed above for DCs, similar questions arise considering tissue-resident macrophage origin and development. Lineage-tracing studies recently revisited their origin and revealed how their maintenance in adult tissues is mostly independent from monocytes and adult definitive hematopoiesis (10). Indeed, tissue-resident macrophages were proposed to develop from a Myb-independent but Sfpi1 (PU.1)-dependent fetal progenitor present in the yolk sac (YS) (5, 6, 15, 98–100) and capable of seeding the developing embryo

and self-renewing during adulthood. This developmental path was first described for microglia, the brain-resident macrophages (98, 99), but still remained elusive for a number of other macrophage populations. Using similar tools, the contribution of YS progenitors to a number of adult tissue-resident macrophage populations was next assessed and only very limited input was found in most tissues tested (101). In parallel, other studies conducted in the lung and skin found that resident alveolar macrophages and Langerhans cells originated from fetal monocytes (99, 102). A recently described hypothesis is now trying to bridge these findings by proposing the existence of erythro-myeloid progenitors (EMP) distinct from hematopoietic stem cells (HSCs), which develop in YS (E8.5) and colonize the fetal liver at E16.5 giving rise to fetal erythrocytes, macrophages, granulocytes, and monocyte (103, 104). Such progenitors would generate microglia early during fetal development and participate to Kupffer cells and Langerhans cells development, but its definitive participation to the generation of other tissue-resident populations, as well as its long-term persistence, still remains to be firmly established. Indeed, a very recent study is now arguing that fetal HSCs, and not YS progenitors or EMPs, give rise to most tissue macrophage populations, except microglia known to originate from YS progenitors other than HSCs (105). This study also highlighted that while most tissue macrophages subsets maintain by self-renewal in the adult, peritoneal, dermal, and colonic residents macrophages needed continuous HSCs input to be maintained during lifetime. Accordingly, gut macrophages, most likely a specific population of macrophage residing in the serosa (106), were shown to derive from HSC-derived circulating monocytes (107). Moreover, blood monocytes can participate to the maintenance of heart macrophages in the adult (108). During inflammation, in addition to tissue-resident macrophages, some macrophages found in tissue differentiate from locally recruited Ly-6C^{hi} monocyte. Such monocyte-derived macrophages reside only for a short period of time in the tissue until inflammation resolves, and are cleared through local cell death (109). Overall, these studies suggest that there is probably more than a single developmental pathway to generate tissue macrophages and to support their self-renewal potential and unique long-term maintenance ability (**Figure 3**).

PATHWAYS ALLOWING FOR TISSUE-RESIDENT MACROPHAGE DEVELOPMENT AND MAINTENANCE

Proceeding along development, Runt-related transcription factor 1 (RUNX1) is required at early stages of myeloid lineage specification and regulates the expression of Sfpi1 (PU.1) which has to be expressed at high levels to allow for development and maintenance of macrophage differentiation (57). One of the most crucial target genes of PU.1 during macrophage development is *Csf1r*, which encodes the receptor for M-CSF and IL-34 (110). Signaling of M-CSFR through either M-CSF or IL-34 allows for the maintenance of tissue-resident macrophages (111, 112). Other TFs required for macrophage development, which co-operate with PU.1 in lineage determination, are AML1 and CCAAT enhancer-binding proteins (C/EBP) (113, 114). Overall, our understanding of the molecular pathways controlling tissue macrophage development in general, as well as their maintenance, remains poorly defined and further studies are need to better characterize how their development is regulated. While for DCs, deletion of a subset might result in minor consequences, macrophages are thought to be critical for the organogenesis and organ homeostasis, therefore deletion of a subset could be deleterious for the life of the individual or only compatible with compensation through alternative subsets or pathways.

TISSUE-RESIDENT MACROPHAGES DIVERSITY AND TISSUE-SPECIFIC TRANSCRIPTION FACTORS CONTROLLING RESIDENT MACROPHAGE DEVELOPMENT AND MAINTENANCE

Our analysis of the transcriptional landscape of tissue-resident macrophages revealed wide heterogeneity across tissues, leading to the definition of population-specific signatures. These specific signatures were recently shown to rely on distinct enhancer landscapes shaped by the tissue microenvironment (115, 116). Using the Immunological Genome database, the reconstruction of lineage-specific regulation from gene-expression profiles across lineages (117) revealed gene modules selectively associated with a single tissue macrophage population (12). Additionally, TFs were predicted to regulate these modules, and thus could potentially influence the development of resident macrophages in a tissue-specific manner (12). Among others, predicted regulators included Spi-C for red pulp macrophages, which confirmed precedent findings (118) and thus validated the predictive power of the algorithm. Indeed, Spi-C is a TF closely related to Sfpi1 and highly expressed in spleen red pulp macrophages compared to other phagocytes (12, 118). Mice deficient for Spi-C lack splenic red pulp macrophages (118), leading to defective red blood cells recycling and iron accumulation in the spleen. At which levels Spi-C acts to control the differentiation and/or survival of red pulp macrophage remains to be determined. LXRα is another TF needed for splenic red pulp macrophage development (119), and whether Spi-C and LXRα interact together in this process is not known. Interestingly, intracellular heme accumulation following erythrocytes uptake induced Spi-C expression by stimulating the degradation of its transcriptional inhibitor Bach1 (118). Thus, heme-induced Spi-C controls the functionality of splenic red pulp macrophages, but also their maintenance albeit by an undetermined mechanism.

Similarly, PPARγ was identified as a regulator for lung macrophages (120). It is a ligand-controlled TF of the nuclear receptor family known for its role in lipid metabolism (121). Previous work has shown that PPARγ expression is important to maintain lung macrophages functionality and surfactant catabolism (122). We reported that conditional deletion of PPARγ in lung macrophages strikingly altered their transcriptome (120). Dysregulated expression of a number of genes involved in lipid metabolism was observed (120), and many of these genes were known targets of the sterol-responsive transcription factor LXR. Accordingly, increased sterol accumulation was observed in lung macrophages lacking PPARγ, as well as decreased expression of genes involved in inflammation and immunity (120). Using a different gene deletion

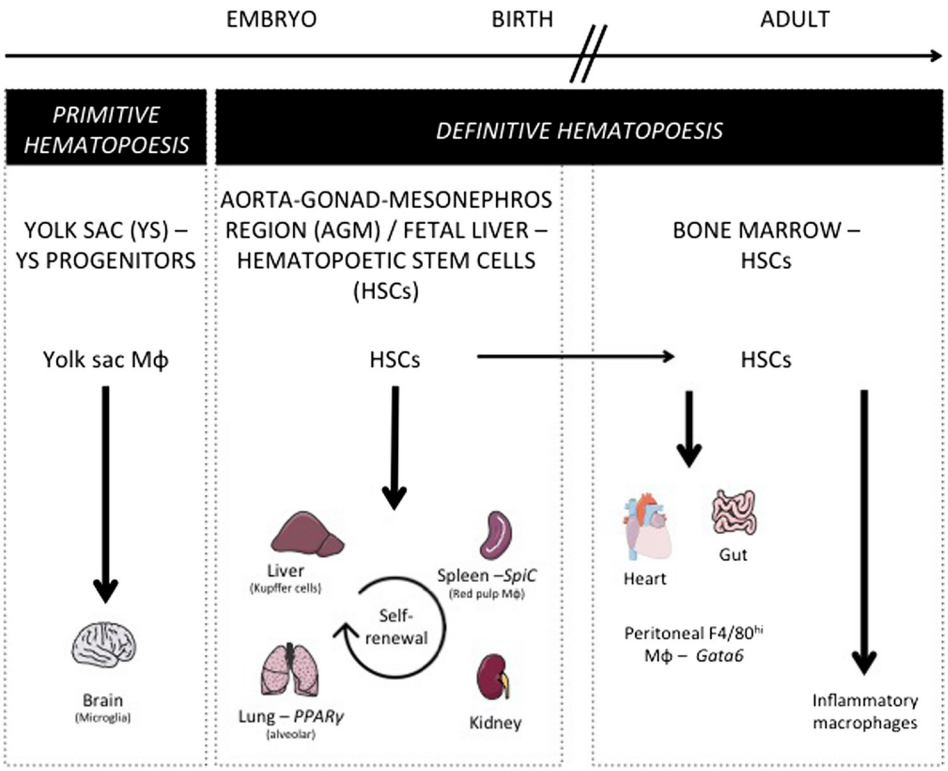

FIGURE 3 | Macrophage ontogeny and requirement on specific transcription factors. This figure shows the progenitors of the different tissue-resident macrophage populations. During embryogenesis, yolk sac progenitors only give rise to microglia. Most tissue-resident macrophages develop before birth and are derived from early hematopoietic stem cells (HSC) present in the aorta–gonad–mesonephros region and in fetal liver. After birth and in the adult mouse, alveolar, splenic, liver, and kidney macrophages maintain their pool by self-renewal, while gut and heart macrophages need a constant replenishment from bone marrow HSC. Additionally, this figure depicts the transcription factors specifically controlling a single resident macrophage population such as Spi-C for splenic red pulp macrophages, PPARγ for alveolar macrophages, and Gata6 for peritoneal F4/80hi macrophages.

approach, it was recently shown that PPARγ could also be key in controlling the development of this subset. Such discrepancy between models might relate to the different temporal induction of the cre expressing strains used in these two studies (123).

Finally, GATA6 was identified as a specific peritoneal macrophage regulator and we observed that its expression was selectively found in F4/80+ peritoneal macrophages across many lineages tested (12), suggesting that it may represent the master regulator of tissue-resident peritoneal macrophages. Interestingly, GATA6 expression by resident peritoneal macrophages was dependent on retinoic acid signaling *in vivo* (12) and mice lacking GATA6 in macrophages, generated by crossing Gata6$^{fl/fl}$ mice with Lyz2-cre, showed a strong reduction in F4/80+ peritoneal macrophages (115–117). Additionally, Th2 inflammation following parasitic infection failed to increase peritoneal macrophage numbers in Lyz2-cre × Gata6$^{fl/fl}$ mice (124), as described for wild-type mice (125). Impaired steady-state numbers of peritoneal macrophages in the absence of GATA6 was accompanied by impaired self-renewal, marked increased in S/G2-M cell cycle phases and accumulation of multinucleated macrophages due to impaired cytokinesis (126). While reduced survival of peritoneal macrophage already explains the strong contraction in their number, impaired cytokinesis will likely further exacerbate the phenotype. GATA6 deficiency in F4/80+ peritoneal macrophages led to the down-regulation of Aspa mRNA, which encodes an aspartoacylase generating acetyl-CoA, a central cellular metabolite, from *N*-acetylaspartate (124). Interestingly, mice lacking Aspa showed reduced F4/80+ peritoneal macrophages. Overall, a tissue-specific transcriptional network driven by GATA6 controls multiple pathways all required for the maintenance of F4/80+ peritoneal macrophages.

CONCLUDING REMARKS

In the past few years, major advances have been made in our understanding how the development of myeloid cells occurs. DNA, RNA protein sequencing and characterization on entire tissues and populations is now a more accessible technology. This combined with improved multicolor flow cytometry and CyTOF technology has allowed us to better understand which TFs identify specific subsets and developmental stages during hematopoietic development (89, 90, 124, 127). However, as it is often the case, the better our analysis tools become, the more complex the picture appears. And despite these advances, we are now starting to perceive how many more gaps need to be filled in order to be able to draw a definitive road map for every MP subset. Significant progress has been made in defining, which TF are needed during DC and macrophage development

in specific tissues. Monocyte development, however, is still elusive and most of the factors identified rather compromise their survival, making hard to discriminate between developmental and survival defects. Moreover, in the past few years, it has become obvious how tissues are able to influence not only the phenotype but also the function of the different subsets. This observation translates in changes in the transcriptional signature, which identifies each subset in a given tissue. Tissue-associated hallmarks have been mostly studied in macrophages, however profound consequences appear to matter also within DC subsets. It will therefore be important to discriminate between tissue- versus subset-specific transcriptional identity, to define intrinsic properties, and functional potential for every subset across and within the different tissues. On the one hand, it is attractive to think that subset specialization similar as for T and innate lymphoid cells is also present within the myeloid compartment. On the other hand, we are aware that myeloid cells are characterized by an elevated intrinsic functional plasticity. Anatomical compartments, pathogen and antigen dose as well as small micro-environmental cues might drastically influence the phenotype, the transcriptional landscape as well as the function of the different subsets during immune responses and we are just starting to explore in depth the complexity of the different subset in response to an immunological insult (90, 127). For DCs, a model has been recently suggested which takes into account the development of a subset with its immunological function. As shown in **Figure 2**, expression of Irf8 and Batf3 is needed in response to pathogens or immunological conditions where IFN-γ is required. On the other side, Irf4 is essential to stimulate Th17, Th2, and IL-22 responses. Within the Irf4 response, Notch2 and Klf4 are specifically required for Th17/IL-22 or Th2 immunity, respectively. The scenario, which appears, is consistent with functional modules of transcription across different cell types, i.e., Klf4 is also necessary for goblet cell development and polarization of M2 macrophages, whereas Notch is required for ILC3 development. Similarly, Nfil3 is important not only for cDC1 but also for NK and ILC1 cells development.

Several TFs that are required for MP development have been characterized; we can draw a map for their temporal requirement along development but for most of them the precise mechanism of action and their targets still need to be identified. Furthermore, since the developmental as well as the functional requirements for a transcriptional pathway are most often shared, caution is necessary to ascribe a specific role to a TF. Recently, a Waddington landscape was suggested to explain the plasticity in DC development (92). A similar concept may reflect and be applied to the entire MP system, where lineage commitment, specific functions, as well as subset identity could depend on the achievement of a threshold of a pool of TFs, rather than a unique master regulator. This concept would explain the so-called "graded-commitment" obtained from barcoding individual progenitors and performing lineage-tracing experiments (128). A second level of complexity is characterized by the fact that multiple subsets share the same TF, though the functional requirements are different. For example, Irf4 seems to regulate migration in skin DCs but not in other peripheral tissues, such as lungs. The functional outcome might be shared; such as in both cases antigen presentation is impaired, however it is important to understand the different requirements depending on the tissue of origin.

The study of MP is characterized by blurry phenotypic boundaries, which do not allow for unequivocal identification of the different subsets. The absence of specific markers leads to the absence of specific genetic tools and sometimes conflicting or unclear results are present in the literature. For lineage-specific deletion within cDC, we still relay on CD11c–cre mice, despite the evident limitations of this model. For monocytes as well as macrophages we lack genetic models, which would allow for selective and specific depletion of inflammatory or patrolling monocytes as well as tissue macrophages. Efforts to generate better lineage-deleter mouse models are therefore required and should be a priority in the next future to better understand the development as well as the contribution of MPs during an immune response.

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REFERENCES

1. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**:571–8. doi:10.1038/nri3712
2. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* (2013) **31**:563–604. doi:10.1146/annurev-immunol-020711-074950
3. Satpathy AT, Wu X, Albring JC, Murphy KM. Re(de)fining the dendritic cell lineage. *Nat Immunol* (2012) **13**:1145–54. doi:10.1038/ni.2467
4. Schlitzer A, McGovern N, Ginhoux F. Dendritic cells and monocyte-derived cells: two complementary and integrated functional systems. *Semin Cell Dev Biol* (2015) **41**:9–22. doi:10.1016/j.semcdb.2015.03.011
5. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**:79–91. doi:10.1016/j.immuni.2012.12.001
6. Varol C, Yona S, Jung S. Origins and tissue-context-dependent fates of blood monocytes. *Immunol Cell Biol* (2009) **87**:30–8. doi:10.1038/icb.2008.90
7. Zigmond E, Varol C, Farache J, Elmaliah E, Satpathy AT, Friedlander G, et al. Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. *Immunity* (2012) **37**:1076–90. doi:10.1016/j.immuni.2012.08.026
8. Geissmann F, Auffray C, Palframan R, Wirrig C, Ciocca A, Campisi L, et al. Blood monocytes: distinct subsets, how they relate to dendritic cells, and their possible roles in the regulation of T-cell responses. *Immunol Cell Biol* (2008) **86**:398–408. doi:10.1038/icb.2008.19
9. Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* (2007) **317**:666–70. doi:10.1126/science.1142883
10. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**:792–804. doi:10.1016/j.immuni.2013.04.004
11. Malissen B, Tamoutounour S, Henri S. The origins and functions of dendritic cells and macrophages in the skin. *Nat Rev Immunol* (2014) **14**:417–28. doi:10.1038/nri3683
12. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**:1118–28. doi:10.1038/ni.2419

13. Belz GT, Nutt SL. Transcriptional programming of the dendritic cell network. *Nat Rev Immunol* (2012) **12**:101–13. doi:10.1038/nri3149
14. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* (2012) **13**:888–99. doi:10.1038/ni.2370
15. Hashimoto D, Miller J, Merad M. Dendritic cell and macrophage heterogeneity in vivo. *Immunity* (2011) **35**:323–35. doi:10.1016/j.immuni.2011.09.007
16. Mildner A, Jung S. Development and function of dendritic cell subsets. *Immunity* (2014) **40**:642–56. doi:10.1016/j.immuni.2014.04.016
17. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* (2007) **7**:19–30. doi:10.1038/nri1996
18. Murphy KM. Transcriptional control of dendritic cell development. *Adv Immunol* (2013) **120**:239–67. doi:10.1016/B978-0-12-417028-5.00009-0
19. Maraskovsky E, Brasel K, Teepe M, Roux ER, Lyman SD, Shortman K, et al. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med* (1996) **184**:1953–62. doi:10.1084/jem.184.5.1953
20. Maraskovsky E, Pulendran B, Brasel K, Teepe M, Roux ER, Shortman K, et al. Dramatic numerical increase of functionally mature dendritic cells in FLT3 ligand-treated mice. *Adv Exp Med Biol* (1997) **417**:33–40. doi:10.1007/978-1-4757-9966-8_6
21. Karsunky H, Merad M, Cozzio A, Weissman IL, Manz MG. Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo. *J Exp Med* (2003) **198**:305–13. doi:10.1084/jem.20030323
22. Naik SH, O'Keeffe M, Proietto A, Hochrein H, Shortman K, Wu L. CD8+, CD8-, and plasmacytoid dendritic cell generation in vitro using flt3 ligand. *Methods Mol Biol* (2010) **595**:167–76. doi:10.1007/978-1-60761-421-0_10
23. Brasel K, De Smedt T, Smith JL, Maliszewski CR. Generation of murine dendritic cells from flt3-ligand-supplemented bone marrow cultures. *Blood* (2000) **96**:3029–39.
24. Gilliet M, Boonstra A, Paturel C, Antonenko S, Xu XL, Trinchieri G, et al. The development of murine plasmacytoid dendritic cell precursors is differentially regulated by Flt3-ligand and granulocyte/macrophage colony-stimulating factor. *J Exp Med* (2002) **195**:953–8. doi:10.1084/jem.20020045
25. McKenna HJ, Stocking KL, Miller RE, Brasel K, De Smedt T, Maraskovsky E, et al. Mice lacking Flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* (2000) **95**:3489–97.
26. Tussiwand R, Onai N, Mazzucchelli L, Manz MG. Inhibition of natural type I IFN-producing and dendritic cell development by a small molecule receptor tyrosine kinase inhibitor with Flt3 affinity. *J Immunol* (2005) **175**:3674–80. doi:10.4049/jimmunol.175.6.3674
27. Manfra DJ, Chen SC, Jensen KK, Fine JS, Wiekowski MT, Lira SA. Conditional expression of murine Flt3 ligand leads to expansion of multiple dendritic cell subsets in peripheral blood and tissues of transgenic mice. *J Immunol* (2003) **170**:2843–52. doi:10.4049/jimmunol.170.6.2843
28. Waskow C, Liu K, Darrasse-Jeze G, Guermonprez P, Ginhoux F, Merad M, et al. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol* (2008) **9**:676–83. doi:10.1038/ni.1615
29. Laouar Y, Welte T, Fu XY, Flavell RA. STAT3 is required for Flt3L-dependent dendritic cell differentiation. *Immunity* (2003) **19**:903–12. doi:10.1016/S1074-7613(03)00332-7
30. Onai N, Obata-Onai A, Tussiwand R, Lanzavecchia A, Manz MG. Activation of the Flt3 signal transduction cascade rescues and enhances type I interferon-producing and dendritic cell development 1. *J Exp Med* (2006) **203**:227–38. doi:10.1084/jem.20051645
31. Rathinam C, Geffers R, Yucel R, Buer J, Welte K, Moroy T, et al. The transcriptional repressor Gfi1 controls STAT3-dependent dendritic cell development and function. *Immunity* (2005) **22**:717–28. doi:10.1016/j.immuni.2005.04.007
32. Hock H, Hamblen MJ, Rooke HM, Schindler JW, Saleque S, Fujiwara Y, et al. Gfi-1 restricts proliferation and preserves functional integrity of haematopoietic stem cells. *Nature* (2004) **431**:1002–7. doi:10.1038/nature02994
33. Karsunky H, Mende I, Schmidt T, Moroy T. High levels of the onco-protein Gfi-1 accelerate T-cell proliferation and inhibit activation induced T-cell death in Jurkat T-cells. *Oncogene* (2002) **21**:1571–9. doi:10.1038/sj.onc.1205216
34. Yucel R, Karsunky H, Klein-Hitpass L, Moroy T. The transcriptional repressor Gfi1 affects development of early, uncommitted c-Kit+ T cell progenitors and CD4/CD8 lineage decision in the thymus. *J Exp Med* (2003) **197**:831–44. doi:10.1084/jem.20021417
35. Kim W, Klarmann KD, Keller JR. Gfi-1 regulates the erythroid transcription factor network through Id2 repression in murine hematopoietic progenitor cells. *Blood* (2014) **124**:1586–96. doi:10.1182/blood-2014-02-556522
36. Li H, Ji M, Klarmann KD, Keller JR. Repression of Id2 expression by Gfi-1 is required for B-cell and myeloid development. *Blood* (2010) **116**:1060–9. doi:10.1182/blood-2009-11-255075
37. Mackarehtschian K, Hardin JD, Moore KA, Boast S, Goff SP, Lemischka IR. Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. *Immunity* (1995) **3**:147–61. doi:10.1016/1074-7613(95)90167-1
38. Adolfsson J, Mansson R, Buza-Vidas N, Hultquist A, Liuba K, Jensen CT, et al. Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* (2005) **121**:295–306. doi:10.1016/j.cell.2005.02.013
39. Wang P, Xue Y, Han Y, Lin L, Wu C, Xu S, et al. The STAT3-binding long noncoding RNA lnc-DC controls human dendritic cell differentiation. *Science* (2014) **344**:310–3. doi:10.1126/science.1251456
40. Wu Y, Smas CM. Wdnm1-like, a new adipokine with a role in MMP-2 activation. *Am J Physiol Endocrinol Metab* (2008) **295**:E205–15. doi:10.1152/ajpendo.90316.2008
41. Tamura T, Tailor P, Yamaoka K, Kong HJ, Tsujimura H, O'Shea JJ, et al. IFN regulatory factor-4 and -8 govern dendritic cell subset development and their functional diversity. *J Immunol* (2005) **174**:2573–81. doi:10.4049/jimmunol.174.5.2573
42. Suzuki S, Honma K, Matsuyama T, Suzuki K, Toriyama K, Akitoyo I, et al. Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha-dendritic cell development. *Proc Natl Acad Sci U S A* (2004) **101**:8981–6. doi:10.1073/pnas.0402139101
43. Yamamoto M, Kato T, Hotta C, Nishiyama A, Kurotaki D, Yoshinari M, et al. Shared and distinct functions of the transcription factors IRF4 and IRF8 in myeloid cell development. *PLoS One* (2011) **6**:e25812. doi:10.1371/journal.pone.0025812
44. Williams JW, Tjota MY, Clay BS, Vander LB, Bandukwala HS, Hrusch CL, et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat Commun* (2013) **4**:2990. doi:10.1038/ncomms3990
45. Li HS, Yang CY, Nallaparaju KC, Zhang H, Liu YJ, Goldrath AW, et al. The signal transducers STAT5 and STAT3 control expression of Id2 and E2-2 during dendritic cell development. *Blood* (2012) **120**:4363–73. doi:10.1182/blood-2012-07-441311
46. Jackson JT, Hu Y, Liu R, Masson F, D'Amico A, Carotta S, et al. Id2 expression delineates differential checkpoints in the genetic program of CD8alpha+ and CD103+ dendritic cell lineages. *EMBO J* (2011) **30**:2690–704. doi:10.1038/emboj.2011.163
47. Nagasawa M, Schmidlin H, Hazekamp MG, Schotte R, Blom B. Development of human plasmacytoid dendritic cells depends on the combined action of the basic helix-loop-helix factor E2-2 and the Ets factor Spi-B. *Eur J Immunol* (2008) **38**:2389–400. doi:10.1002/eji.200838470
48. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development 1. *Cell* (2008) **135**:37–48. doi:10.1016/j.cell.2008.09.016
49. Spits H, Couwenberg F, Bakker AQ, Weijer K, Uittenbogaart CH. Id2 and Id3 inhibit development of CD34(+) stem cells into predendritic cell (pre-DC)2 but not into pre-DC1. Evidence for a lymphoid origin of pre-DC2. *J Exp Med* (2000) **192**:1775–84. doi:10.1084/jem.192.12.1775
50. Hacker C, Kirsch RD, Ju XS, Hieronymus T, Gust TC, Kuhl C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat Immunol* (2003) **4**:380–6. doi:10.1038/ni903
51. Ghosh HS, Cisse B, Bunin A, Lewis KL, Reizis B. Continuous expression of the transcription factor e2-2 maintains the cell fate of mature plasmacytoid dendritic cells. *Immunity* (2010) **33**:905–16. doi:10.1016/j.immuni.2010.11.023
52. Ghosh HS, Ceribelli M, Matos I, Lazarovici A, Bussemaker HJ, Lasorella A, et al. ETO family protein Mtg16 regulates the balance of dendritic cell subsets by repressing Id2. *J Exp Med* (2014) **211**:1623–35. doi:10.1084/jem.20132121
53. Kashiwada M, Pham NL, Pewe LL, Harty JT, Rothman PB. NFIL3/E4BP4 is a key transcription factor for CD8{alpha}+ dendritic cell development. *Blood* (2011) **117**:6193–7. doi:10.1182/blood-2010-07-295873

54. Onai N, Kurabayashi K, Hosoi-Amaike M, Toyama-Sorimachi N, Matsushima K, Inaba K, et al. A clonogenic progenitor with prominent plasmacytoid dendritic cell developmental potential. *Immunity* (2013) **38**:943–57. doi:10.1016/j.immuni.2013.04.006
55. Liu K, Victora GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, et al. In vivo analysis of dendritic cell development and homeostasis. *Science* (2009) **324**:392–7. doi:10.1126/science.1170540
56. Kurotaki D, Yamamoto M, Nishiyama A, Uno K, Ban T, Ichino M, et al. IRF8 inhibits C/EBPalpha activity to restrain mononuclear phagocyte progenitors from differentiating into neutrophils. *Nat Commun* (2014) **5**:4978. doi:10.1038/ncomms5978
57. Kueh HY, Champhekar A, Nutt SL, Elowitz MB, Rothenberg EV. Positive feedback between PU.1 and the cell cycle controls myeloid differentiation. *Science* (2013) **341**:670–3. doi:10.1126/science.1240831
58. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* (2002) **109**:625–37. doi:10.1016/S0092-8674(02)00754-7
59. Naik SH, Proietto AI, Wilson NS, Dakic A, Schnorrer P, Fuchsberger M, et al. Cutting edge: generation of splenic CD8+ and CD8- dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. *J Immunol* (2005) **174**:6592–7. doi:10.4049/jimmunol.174.11.6592
60. Naik SH, Metcalf D, van Nieuwenhuijze A, Wicks I, Wu L, O'Keeffe M, et al. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat Immunol* (2006) **7**:663–71. doi:10.1038/ni1340
61. Liu K, Waskow C, Liu X, Yao K, Hoh J, Nussenzweig M. Origin of dendritic cells in peripheral lymphoid organs of mice. *Nat Immunol* (2007) **8**:578–83. doi:10.1038/ni1462
62. Grajales-Reyes GE, Iwata A, Albring J, Wu X, Tussiwand R, Kc W, et al. Batf3 maintains autoactivation of Irf8 for commitment of a CD8alpha(+) conventional DC clonogenic progenitor. *Nat Immunol* (2015) **16**:708–17. doi:10.1038/ni.3197
63. Schlitzer A, Sivakamasundari V, Chen J, Sumatoh HR, Schreuder J, Lum J, et al. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. *Nat Immunol* (2015) **16**:718–28. doi:10.1038/ni.3200
64. Meredith MM, Liu K, Kamphorst AO, Idoyaga J, Yamane A, Guermonprez P, et al. Zinc finger transcription factor zDC is a negative regulator required to prevent activation of classical dendritic cells in the steady state. *J Exp Med* (2012) **209**:1583–93. doi:10.1084/jem.20121003
65. Reizis B. Classical dendritic cells as a unique immune cell lineage. *J Exp Med* (2012) **209**:1053–6. doi:10.1084/jem.20121038
66. Satpathy AT, Kc W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med* (2012) **209**:1135–52. doi:10.1084/jem.20120030
67. Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* (2013) **154**:843–58. doi:10.1016/j.cell.2013.07.014
68. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* (2008) **322**:1097–100. doi:10.1126/science.1164206
69. Edelson BT, Kc W, Juang R, Kohyama M, Benoit LA, Klekotka PA, et al. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J Exp Med* (2010) **207**:823–36. doi:10.1084/jem.20091627
70. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* (2009) **206**:3115–30. doi:10.1084/jem.20091756
71. Seillet C, Jackson JT, Markey KA, Hill GR, Macdonald KP, Nutt SL, et al. CD8alpha+ DCs can be induced in the absence of transcription factors Id2, Nfil3 and Batf3. *Blood* (2013) **121**:1574–83. doi:10.1182/blood-2012-07-445650
72. Tussiwand R, Lee WL, Murphy TL, Mashayekhi M, Wumesh KC, Albring JC, et al. Compensatory dendritic cell development mediated by BATF-IRF interactions. *Nature* (2012) **490**:502–7. doi:10.1038/nature11531
73. Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hagerbrand K, Marsal J, et al. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* (2013) **38**:958–69. doi:10.1016/j.immuni.2013.03.009
74. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b(+) dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* (2013) **38**:970–83. doi:10.1016/j.immuni.2013.04.011
75. Vander LB, Khan AA, Hackney JA, Agrawal S, Lesch J, Zhou M, et al. Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. *Nat Immunol* (2014) **15**:161–7. doi:10.1038/ni.2795
76. Zhou Q, Ho AW, Schlitzer A, Tang Y, Wong KH, Wong FH, et al. CD11b+ lung dendritic cells orchestrate Th2 immunity to *Blomia tropicalis*. *J Immunol* (2014) **193**:496–509. doi:10.4049/jimmunol.1303138
77. Gao Y, Nish SA, Jiang R, Hou L, Licona-Limon P, Weinstein JS, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* (2013) **39**:722–32. doi:10.1016/j.immuni.2013.08.028
78. Bajana S, Roach K, Turner S, Paul J, Kovats S. IRF4 promotes cutaneous dendritic cell migration to lymph nodes during homeostasis and inflammation. *J Immunol* (2012) **189**:3368–77. doi:10.4049/jimmunol.1102613
79. Burkly L, Hession C, Ogata L, Reilly C, Marconi LA, Olson D, et al. Expression of relB is required for the development of thymic medulla and dendritic cells. *Nature* (1995) **373**:531–6. doi:10.1038/373531a0
80. Wu L, D'Amico A, Winkel KD, Suter M, Lo D, Shortman K. RelB is essential for the development of myeloid-related CD8alpha– dendritic cells but not of lymphoid-related CD8alpha+ dendritic cells. *Immunity* (1998) **9**:839–47. doi:10.1016/S1074-7613(00)80649-4
81. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J Exp Med* (2007) **204**:1653–64. doi:10.1084/jem.20062648
82. Cheng P, Nefedova Y, Miele L, Osborne BA, Gabrilovich D. Notch signaling is necessary but not sufficient for differentiation of dendritic cells. *Blood* (2003) **102**:3980–8. doi:10.1182/blood-2003-04-1034
83. Satpathy AT, Briseno CG, Lee JS, Ng D, Manieri NA, Kc W, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol* (2013) **14**:937–48. doi:10.1038/ni.2679
84. Tussiwand R, Everts B, Grajales-Reyes GE, Kretzer NM, Iwata A, Bagaitkar J, et al. Klf4 expression in conventional dendritic cells is required for T helper 2 cell responses. *Immunity* (2015) **42**:916–28. doi:10.1016/j.immuni.2015.04.017
85. Kurotaki D, Osato N, Nishiyama A, Yamamoto M, Ban T, Sato H, et al. Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation. *Blood* (2013) **121**:1839–49. doi:10.1182/blood-2012-06-437863
86. Dicken J, Mildner A, Leshkowitz D, Touw IP, Hantisteanu S, Jung S, et al. Transcriptional reprogramming of CD11b+Esam(hi) dendritic cell identity and function by loss of Runx3. *PLoS One* (2013) **8**:e77490. doi:10.1371/journal.pone.0077490
87. Satpathy AT, Briseño CG, Cai X, Michael DG, Chou C. Hsiung S, et al. Runx1 and Cbfβ regulate the development of dendritic cell precursors by restricting granulocyte/macrophage lineages. *Blood* (2014) **123**:2968–77. doi:10.1182/blood-2013-11-539643
88. Kobayashi T, Walsh PT, Walsh MC, Speirs KM, Chiffoleau E, King CG, et al. TRAF6 is a critical factor for dendritic cell maturation and development. *Immunity* (2003) **19**:353–63. doi:10.1016/S1074-7613(03)00230-9
89. Amit I, Garber M, Chevrier N, Leite AP, Donner Y, Eisenhaure T, et al. Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* (2009) **326**:257–63. doi:10.1126/science.1179050
90. Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science* (2014) **343**:776–9. doi:10.1126/science.1247651
91. Mildner A, Chapnik E, Manor O, Yona S, Kim KW, Aychek T, et al. Mononuclear phagocyte miRNome analysis identifies miR-142 as critical regulator of murine dendritic cell homeostasis. *Blood* (2013) **121**:1016–27. doi:10.1182/blood-2012-07-445999
92. Paul F, Amit I. Plasticity in the transcriptional and epigenetic circuits regulating dendritic cell lineage specification and function. *Curr Opin Immunol* (2014) **30**:1–8. doi:10.1016/j.coi.2014.04.004
93. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, et al. Notch2 receptor signaling controls functional differentiation of dendritic

cells in the spleen and intestine. *Immunity* (2011) **35**:780–91. doi:10.1016/j.immuni.2011.08.013
94. Watchmaker PB, Lahl K, Lee M, Baumjohann D, Morton J, Kim SJ, et al. Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. *Nat Immunol* (2014) **15**:98–108. doi:10.1038/ni.2768
95. Terry RL, Miller SD. Molecular control of monocyte development. *Cell Immunol* (2014) **291**:16–21. doi:10.1016/j.cellimm.2014.02.008
96. Friedman AD. Transcriptional control of granulocyte and monocyte development. *Oncogene* (2007) **26**:6816–28. doi:10.1038/sj.onc.1210764
97. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* (2013) **14**:821–30. doi:10.1038/ni.2638
98. Schulz C, Gomez PE, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**:86–90. doi:10.1126/science.1219179
99. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) **330**:841–5. doi:10.1126/science.1194637
100. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science* (2010) **327**:656–61. doi:10.1126/science.1178331
101. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* (2014) **40**:91–104. doi:10.1016/j.immuni.2013.11.019
102. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* (2013) **210**:1977–92. doi:10.1084/jem.20131199
103. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2015) **518**:547–51. doi:10.1038/nature13989
104. Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med* (2012) **209**:1167–81. doi:10.1084/jem.20120340
105. Sheng J, Ruedl C, Karjalainen K. Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. *Immunity* (2015) **43**:382–93. doi:10.1016/j.immuni.2015.07.016
106. Muller PA, Koscso B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* (2014) **158**:300–13. doi:10.1016/j.cell.2014.04.050
107. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) **15**:929–37. doi:10.1038/ni.2967
108. Molawi K, Wolf Y, Kandalla PK, Favret J, Hagemeyer N, Frenzel K, et al. Progressive replacement of embryo-derived cardiac macrophages with age. *J Exp Med* (2014) **211**:2151–8. doi:10.1084/jem.20140639
109. Gautier EL, Ivanov S, Lesnik P, Randolph GJ. Local apoptosis mediates clearance of macrophages from resolving inflammation in mice. *Blood* (2013) **122**:2714–22. doi:10.1182/blood-2013-01-478206
110. Chihara T, Suzu S, Hassan R, Chutiwitoonchai N, Hiyoshi M, Motoyoshi K, et al. IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation. *Cell Death Differ* (2010) **17**:1917–27. doi:10.1038/cdd.2010.60
111. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-derived interleukin-34 controls the development and maintenance of Langerhans cells and the maintenance of microglia. *Immunity* (2012) **37**:1050–60. doi:10.1016/j.immuni.2012.11.001
112. Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat Immunol* (2012) **13**:753–60. doi:10.1038/ni.2360
113. Zhang P, Iwasaki-Arai J, Iwasaki H, Fenyus ML, Dayaram T, Owens BM, et al. Enhancement of hematopoietic stem cell repopulating capacity and self-renewal in the absence of the transcription factor C/EBP alpha. *Immunity* (2004) **21**:853–63. doi:10.1016/j.immuni.2004.11.006
114. Zhang DE, Hetherington CJ, Meyers S, Rhoades KL, Larson CJ, Chen HM, et al. CCAAT enhancer-binding protein (C/EBP) and AML1 (CBF alpha2) synergistically activate the macrophage colony-stimulating factor receptor promoter. *Mol Cell Biol* (1996) **16**:1231–40.
115. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**:1327–40. doi:10.1016/j.cell.2014.11.023
116. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
117. Jojic V, Shay T, Sylvia K, Zuk O, Sun X, Kang J, et al. Identification of transcriptional regulators in the mouse immune system. *Nat Immunol* (2013) **14**:633–43. doi:10.1038/ni.2587
118. Haldar M, Kohyama M, So AY, Kc W, Wu X, Briseno CG, et al. Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. *Cell* (2014) **156**:1223–34. doi:10.1016/j.cell.2014.01.069
119. A-Gonzalez N, Guillen JA, Gallardo G, Diaz M, de la Rosa JV, Hernandez IH, et al. The nuclear receptor LXRalpha controls the functional specialization of splenic macrophages. *Nat Immunol* (2013) **14**:831–9. doi:10.1038/ni.2622
120. Gautier EL, Chow A, Spanbroek R, Marcelin G, Greter M, Jakubzick C, et al. Systemic analysis of PPARgamma in mouse macrophage populations reveals marked diversity in expression with critical roles in resolution of inflammation and airway immunity. *J Immunol* (2012) **189**:2614–24. doi:10.4049/jimmunol.1200495
121. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem* (2008) **77**:289–312. doi:10.1146/annurev.biochem.77.061307.091829
122. Baker AD, Malur A, Barna BP, Ghosh S, Kavuru MS, Malur AG, et al. Targeted PPAR{gamma} deficiency in alveolar macrophages disrupts surfactant catabolism. *J Lipid Res* (2010) **51**:1325–31. doi:10.1194/jlr.M001651
123. Schneider C, Nobs SP, Kurrer M, Rehrauer H, Thiele C, Kopf M. Induction of the nuclear receptor PPAR-gamma by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages. *Nat Immunol* (2014) **15**:1026–37. doi:10.1038/ni.3005
124. Gautier EL, Ivanov S, Williams JW, Huang SC, Marcelin G, Fairfax K, et al. Gata6 regulates aspartoacylase expression in resident peritoneal macrophages and controls their survival. *J Exp Med* (2014) **211**:1525–31. doi:10.1084/jem.20140570
125. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, Van Rooijen N, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science* (2011) **332**:1284–8. doi:10.1126/science.1204351
126. Rosas M, Davies LC, Giles PJ, Liao CT, Kharfan B, Stone TC, et al. The transcription factor Gata6 links tissue macrophage phenotype and proliferative renewal. *Science* (2014) **344**:645–8. doi:10.1126/science.1251414
127. Becher B, Schlitzer A, Chen J, Mair F, Sumatoh HR, Teng KW, et al. High-dimensional analysis of the murine myeloid cell system. *Nat Immunol* (2014) **15**:1181–9. doi:10.1038/ni.3006
128. Naik SH, Perie L, Swart E, Gerlach C, van Rooij N, de Boer RJ, et al. Diverse and heritable lineage imprinting of early haematopoietic progenitors. *Nature* (2013) **496**:229–32. doi:10.1038/nature12013

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Fate mapping of dendritic cells

Mateusz Pawel Poltorak and Barbara Ursula Schraml *

Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany

Dendritic cells (DCs) are a heterogeneous group of mononuclear phagocytes with versatile roles in immunity. They are classified predominantly based on phenotypic and functional properties, namely their stellate morphology, expression of the integrin CD11c, and major histocompatibility class II molecules, as well as their superior capacity to migrate to secondary lymphoid organs and stimulate naïve T cells. However, these attributes are not exclusive to DCs and often change within inflammatory or infectious environments. This led to debates over cell identification and questioned even the mere existence of DCs as distinct leukocyte lineage. Here, we review experimental approaches taken to fate map DCs and discuss how these have shaped our understanding of DC ontogeny and lineage affiliation. Considering the ontogenetic properties of DCs will help to overcome the inherent shortcomings of purely phenotypic- and function-based approaches to cell definition and will yield a more robust way of DC classification.

Keywords: dendritic cell, ontogeny, fate mapping, lineage tracing, mononuclear phagocyte

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***Correspondence:**
Barbara Ursula Schraml,
Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Trogerstraße 30, Munich 81675, Germany
barbara.schraml@tum.de

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Introduction

Dendritic cells (DCs) were originally identified in mouse spleen for their unique stellate morphology, their ability to adhere to certain glass surfaces and their superior capacity to activate naïve T lymphocytes that distinguished them from macrophages (MØs) (1–3). Mostly for historical reasons, DCs are considered part of the mononuclear phagocyte (MP) system, which groups all highly phagocytic cells derived from monocytes or their precursors based on the premise that tissue MØs arise from monocytes (4–9). This presumed relatedness of DCs, monocytes, and MØs coupled to the lack of reliable ways to distinguish MP subtypes has caused continuous debates over accurate cell-type identification and has led some to question whether DCs in fact constitute an independent cell lineage (6, 7, 10–14). However, today we have conclusive evidence demonstrating that DCs, monocytes, and MØs have distinct cellular origin and we further distinguish plasmacytoid DCs (pDCs) from two subsets of so-called conventional or classical DCs (cDCs) based on unique developmental requirements (7, 15–19). Nonetheless, DCs remain defined based on phenotypic and functional properties that often overlap with those of monocytes or MØs (19), although some have suggested a shift in paradigm toward a nomenclature that takes cell ontogeny into account (6, 7, 10).

Dendritic cells are generally identified by their high expression of major histocompatibility complex class II molecules (MHCII) and of the integrin CD11c, as well as their superior capacity to migrate from non-lymphoid to lymphoid organs and stimulate naïve T cells (3, 20–22). However, these characteristics are not absolute and can change in situations of inflammation or infection, thus complicating cell identification (6, 7, 23, 24). For instance, CD11c, considered the hallmark surface marker of DCs, is also found on B, T, and NK cells as well as some monocytes, MØs, and eosinophils (25–32). Dendritic protrusions have also been observed in some MØs and T cells (33–35). Further, surface markers, such as F4/80, CD14, or CD64 (Fc-gamma receptor 1), generally associated with monocytes or MØs can be found on DCs (36–38). One might argue that the most defining feature of DCs is their ability to activate T cells, however such definition discounts the fact that DCs potently

regulate innate immune responses independent of their ability to migrate to lymphoid organs or stimulate T cells (39–44). Conversely, non-DCs can carry antigen to lymph nodes and activate naïve T cells in some instances (45–47).

Therefore, morphological and functional properties, as well as the expression of surface markers are insufficient to clearly distinguish DCs from monocytes and MØs, raising the necessity to find a more robust way of cell identification. Recent studies in mouse and human indicate that DCs, MØs, and monocytes have unique ontogenetic properties and thus can be considered distinct cell lineages (36, 48–54). Here, we review approaches that have been employed to track and define the progeny of DC precursors *in vivo* and discuss how such "fate mapping" approaches have improved our understanding of DC heterogeneity and ontogeny. These studies lay the foundation for moving toward cell ontogeny as a major lineage-determining criterion, which will allow for a more reliable and precise classification of DCs and DC subsets.

DC Development

Dendritic cells are short-lived and their maintenance relies on constant replenishment from bone marrow progenitors that originate from hematopoietic stem cells (HSCs) (19, 55). In the classic model of DC development monocytes and DCs arise from bi-potent progenitors, so-called MØ and DC progenitors (MDPs) (**Figure 1**) (56). MDPs further give rise to common DC progenitors (CDPs) restricted to the generation of pDCs and cDCs (**Figure 1**) (57, 58). pDCs terminally differentiate in the bone marrow, thus exit the bone marrow as fully developed cells and reach peripheral organs via the blood stream (**Figure 1**) (15, 59). In contrast, cDCs arise from another developmental intermediate termed pre-DC, which exits the bone marrow and migrates through the blood to seed lymphoid and non-lymphoid tissues (60, 61). There, pre-DCs terminally differentiate into cDCs, including the main CD11b$^-$ and CD11b$^+$ subtypes (**Figure 1**) (60–63). In lymphoid tissues these are CD8α$^+$CD11b$^-$ and CD11b$^+$ resident cDCs, whereas in non-lymphoid tissues they comprise CD103$^+$CD11b$^-$ and CD11b$^+$ migratory cDCs (3, 60–63). Like pDCs, monocytes complete their development in the bone marrow but in tissues they differentiate into cells with DC- or MØ-like features (**Figure 1**) (23, 24, 64, 65). This plasticity is remarkably prominent in inflammatory or infectious environments, when monocyte-derived cells with qualities of DCs have been referred to as TNF-α/iNOS-producing DCs (Tip-DCs), monocyte-derived DCs (mo-DCs), and/or inflammatory DCs (23, 24, 64, 65).

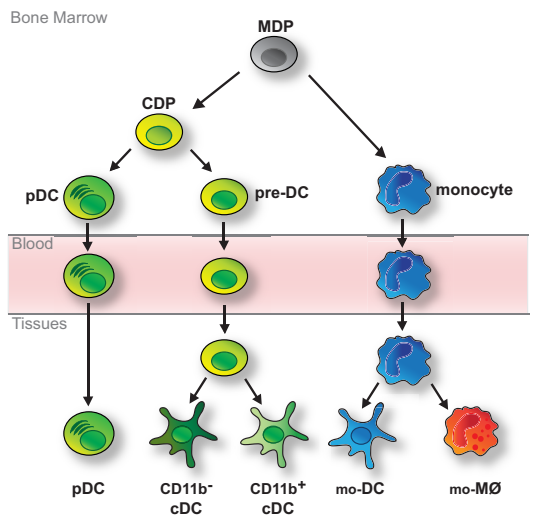

FIGURE 1 | Classic model of DC development. DCs and monocytes are ancestrally related and arise from bi-potential MDPs residing in the bone marrow. MDPs further differentiate into monocytes and CDPs, which are restricted to the generation of various types of DCs. CDPs give rise to pDCs, which fully develop in the bone marrow, and pre-DCs, which migrate through the blood to tissues, where they fully differentiate into CD11b$^-$ (including CD8α$^+$ cDCs in lymphoid tissue and migratory CD103$^+$ cDCs in non-lymphoid tissue) and CD11b$^+$ cDCs. Monocytes complete their development in the bone marrow and reach peripheral tissues via the bloodstream. There they further differentiate into monocyte-derived DCs (mo-DCs) or MØs (mo-MØs) in response to environmental cues.

Although most of our knowledge concerning DC development is derived from mouse studies, developmental parallels have been observed in other species (66–73). Especially the identification of putative equivalent DC progenitor populations in human holds promise for future research (72, 73). Yet, some uncertainties remain. Common lymphoid progenitors (CLPs) can give rise to DC descendants upon adoptive transfer (74), although it is now thought that DCs originate predominantly from myeloid progenitors (75, 76). Nonetheless, some pDCs, but not cDCs, show evidence of VDJ gene rearrangements, potentially indicating lymphoid lineage heritage (15, 59, 77). However, it remains unclear whether evidence of *Rag* gene expression history necessarily means that pDCs have dual lymphoid and myeloid origin. Contrary to the dogma that monocytes and DCs share a common immediate ancestor, recent data suggest that lineage divergence of HSC-derived myeloid cells occurs much earlier than previously predicted and that monocytes and DCs might arise independent of a bi-potential developmental intermediate (49, 78, 79). Elucidating such unresolved aspects pertaining to DC ontogeny may solve uncertainties in determining lineage affiliation, which, in turn, will aid to further decipher the unique functions of DCs in immunity.

Fate Mapping

Understanding cell development requires models with which the relationship of a precursor cell and its progeny can be defined *in vivo*. Such "fate mapping" can be achieved in various ways and relies on the selective labeling of the cell(s) of interest so that consequently the development of the marked cell can be followed in its natural environment (80). Tracing progenitors *in vivo* also offers the possibility to determine the fate of populations when lineage affiliation is most heavily debated, namely following experimental manipulation to generate conditions of inflammation or infection. While most fate mapping strategies follow the progeny of bulk cell populations, recently developed techniques have enabled the tracing of single cells, thus providing valuable information regarding their developmental potential at the clonal level (80, 81). In all fate mapping experiments, it is important to consider that their

interpretation is dependent on the use of select, faithful and stable markers (82).

Precursor Transfers

The transfer of purified and pre-marked precursor cells into congenic recipients is the most accessible form of fate mapping as a variety of labeling options can be used to distinguish between donor and host cells (**Figure 2A**) (80). As a result, precursor transfers are commonly used to study cell development and lineage relationships and remain a standard protocol for defining the stemness of progenitor cells (80). Such experiments rely on the ability to purify sufficient precursors that, after cell isolation, retain the capacity to home to the appropriate anatomical niche and expand sufficiently into detectable progeny. To circumvent such limitations transfer studies are often combined with protocols to induce leukopenia, such as irradiation, in order to increase the niche available for cell engraftment (**Figure 2A**) (80). However, these manipulations can alter developmental signals, which, in turn, might impact on the interpretation of results (18, 54, 83). To best mimic the endogenous cellular environment, progenitors have been returned directly to their organs of origin, for instance by intra-bone injection (84).

The DC progenitors MDP, CDP, and pre-DC were in part defined by assessing their developmental potential after adoptive transfer into mice (56–58, 60, 61, 84–86). In such experiments, MDPs give rise to DCs and monocytes, whereas CDPs and pre-DCs are restricted to the generation of DCs but do not generate monocytes or other leukocyte lineages (56–58, 61, 84–86). In combination with experiments assessing the differentiation potential of single progenitors *in vitro* (56–58), these studies have significantly shaped our view of DC development (**Figure 1**). Surprisingly, the existence of MDP as a bi-potential intermediate for DCs and monocytes has recently been questioned when single CX_3CR1^+ MDPs were unable to generate both DCs and monocytes upon differentiation *in vitro* (78). The authors further found that adoptively transferred CX_3CR1^+ MDPs, not only gave rise to DCs and monocytes but also neutrophils (78). However, such multi-potency of MDPs was not observed in earlier studies (52, 56, 61, 85, 86) and is not evident in genetic CX_3CR1 fate mapping experiments (50). It is possible that these discrepancies may be explained by experimental variation such as differences in cell isolation, the timing of analysis or variances in the niche available for cell engraftment following irradiation (18, 54, 83). In light of these results it is noteworthy, however, that upon adoptive transfer MDPs exhibit pDC potential only in some studies (52, 86) but not others (56, 85), whereas the presumed downstream CDPs produce both pDCs and cDCs (57, 58). Taken together these experiments raise some doubt about the existence of a MDP as a key developmental intermediate for monocytes, cDCs, and pDCs. However, resolving this matter will require the use of better models to trace single cells *in vivo* as experiments relying on the isolation and analysis of bulk progenitor populations are inherently prone to disparities in gating strategy or cell purity.

In DC ontogeny, these issues are augmented because MDP and CDP exhibit substantial phenotypic overlap: both lack lineage-defining markers, are characterized by expression of CX_3CR1, CD115 (M-CSFR, Csf1R) as well as CD135 (FMS-like tyrosine kinase 3, FLT3) and, until recently, CDP could only be distinguished from MDP by lower expression of the receptor tyrosine kinase CD117 (c-kit) (56–58, 61, 86). We have recently found that the C-type lectin receptor DNGR-1 (Clec9a) marks cells resembling CDPs (36). Surprisingly, upon adoptive transfer, $DNGR\text{-}1^+CD115^+$ progenitors exhibit cDC-restricted differentiation potential and do not generate pDCs (36), suggesting that DNGR-1 marks cDC-restricted progenitors. These data are in line with a recent study demonstrating a strong bias for $CD115^+$ CDPs to generate cDCs, whereas pDCs arise predominantly from CD115 negative cells (79). Therefore, cDCs and pDCs appear to have distinct developmental intermediates that can be distinguished by expression of CD115 (79) and DNGR-1 (36). Since $CD115^+$ CDPs presumably express DNGR-1 (36), it is unclear why some $CD115^+$ CDPs show combined cDC and pDC potential in clonal assays (57, 58, 79). It is possible that antibody-mediated triggering of DNGR-1 or growth factor receptors, such as CD115, during cell isolation skews DC differentiation toward a particular DC sub-lineage in an unforeseeable manner. The developmental potential of progenitors may also be influenced by the specific culture conditions used (78) or DCs could exhibit a degree of developmental plasticity (87). Nonetheless, the existence of a putative intermediate monocyte-restricted progenitor downstream of MDP (common monocyte progenitor, cMoP) (52) alongside the aforementioned pDC- and cDC-restricted progenitors supports a model in which monocytes, cDCs, and pDCs develop independently. The genuine point of lineage divergence, however, remains to be determined.

Questions regarding the lineage affiliation of DCs have been muddled significantly by the developmental plasticity of monocytes (6, 24). The phenotypic transformation of monocytes into DC-like cells is most prominent in inflamed environments (8, 19, 23, 24). It can also be mimicked *in vitro* by culturing monocytes in the presence of GM-CSF (granulocyte-macrophage colony-stimulating factor) ± IL-4 (Interleukin-4) (88, 89). However, *in vivo* the inflammation-induced differentiation of monocytes into cells with attributes of DCs appears GM-CSF-independent (90), highlighting that the developmental requirements underlying this phenotypic conversion *in vitro* might differ from those involved *in vivo*. In the absence of experimentally induced infection or inflammation, adoptively transferred monocytes readily acquire CD11c and MHCII expression as well as functional features of DCs in non-lymphoid tissues (91–95). This phenotypic conversion is also observed after adoptive transfer into unirradiated hosts, which most closely mimics steady-state conditions (63). In contrast, transferred monocytes do not generate DCs in lymphoid organs, even if the niche for engraftment is opened by depletion of $CD11c^+$ cells (84). Importantly, in non-lymphoid tissues monocytes exclusively generate $CD11b^+$, but not $CD103^+CD11b^-$ cells, which is in contrast to CDPs and pre-DCs that generate $CD11b^+$ as well as $CD103^+CD11b^-$ cDCs (63, 91–95). Therefore, $CD11c^+MHCII^+CD11b^+$ cells in non-lymphoid tissues appear to constitute a population of mixed cellular origin that can arise from monocytic progenitors as well as pre-DCs. Adoptive transfer experiments do not allow to determine the relative contribution of each progenitor to this population,

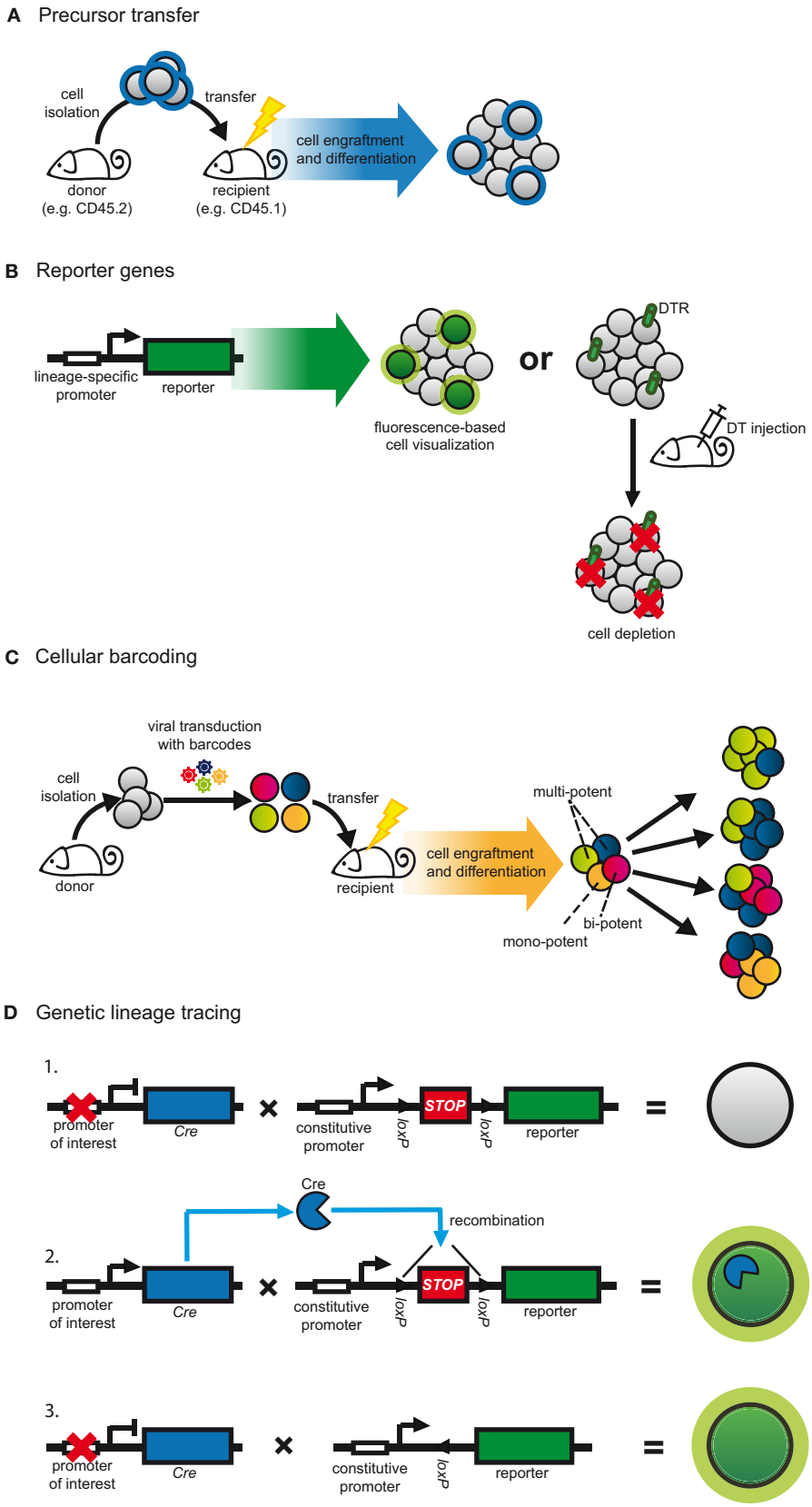

FIGURE 2 | Strategies to fate map DCs.

(Continued)

FIGURE 2 | Continued
(A) Progenitors are adoptively transferred to assess their differentiation in the physiological context. Graft-derived cells are distinguished from host cells based on pre-defined labels, for instance congenic markers. This method is often combined with strategies to increase the niche available for cell engraftment, such as irradiation. **(B)** In transgenic approaches, lineage-restricted promoters can be used to drive a reporter gene. Target cell populations can be visualized by the expression of fluorescent proteins or can be depleted. In the latter case, cell-restricted expression of DTR allows for conditional cell ablation following DT injection. **(C)** Progenitors are transduced *in vitro* with semi-random DNA sequences (barcodes) by retro- or lentiviral vectors and subsequently transferred into irradiated congenic recipients. After differentiation, cell progeny are analyzed for their barcode repertoire using deep sequencing or microarray. The representation of a given barcode in multiple cell populations indicates multi-potency of the transferred cell. **(D)** Expression of Cre recombinase is driven by a lineage or cell-specific promoter. Additionally, a reporter gene, usually a fluorescent protein, is placed under control of a constitutive promoter. Expression of the reporter is blocked by inserting a *loxP*-flanked *STOP* cassette (1). Cre expression leads to excision of the *STOP* cassette resulting in expression of the reporter gene (2). Since the promoter-driving reporter gene expression is constitutively active, the target cell is irreversibly labeled irrespective of continuous Cre expression (3).

although surrogate markers such as CD64 or Mar-1 can serve to distinguish monocyte-derived cells from bona fide pre-DC-derived cDCs (46, 93, 94).

Notably, in irradiated hosts transferred monocytes can also generate $CD11c^+MHCII^+$ cells of the epidermis, which resemble Langerhans cells (LCs) (96–99). LCs exhibit many phenotypic and functional features of DCs, such as the capacity to migrate to lymphoid organs and stimulate naïve T cells, and have long been considered a prototypical DC population (96–99). However, we now realize that the majority of LCs is established before birth and maintained under steady-state conditions by self-renewal from local progenitors (96, 97, 99–102). These properties thus ontogenetically separate LCs from bone marrow-derived DCs or monocyte-derived cells. Moreover, monocytes may not necessarily adopt features of DCs or MØs upon entry into tissues, as a recent study indicates that monocyte can also exist in tissues without further differentiation (45). When considering this immense plasticity it will be crucial to elucidate the environmental cues that shape the diverse fates of monocytes to further dissect the full functional spectrum of monocytes and monocyte-derived cells.

Lineage Restricted Reporters

When the availability of isolatable progenitor cells is limiting and when populations are ontogenetically heterogeneous or might be influenced by alterations in their surroundings, determining lineage affiliation requires models to trace cells directly in their natural environment. One way to achieve this is by engineering models in which lineage-restricted promoters or genetic elements drive the expression of reporter genes (**Figure 2B**) (80, 82, 103). It is important to bear in mind that such experiments assume that the expression of the selected marker is restricted to the cell lineage in question and therefore, the choice of stable and specific markers is essential (80, 82, 103). Additionally, the genetic elements used to drive expression of the reporter must faithfully mimic endogenous gene expression (80, 82, 103).

Genetic elements of the *Itgax* gene, which encodes CD11c, have extensively been used to generate reporters to study DCs (82, 103). As such, transgenic mice in which the CD11c promoter drives the expression of fluorescent proteins (**Figure 2B**) have been key to visualizing the distribution and cellular interactions of DCs in a variety of tissues, including lymphoid organs, heart, lung, and skin (103–107). But fate mapping can also be achieved by cell deletion. Transgenic expression of primate diphtheria toxin receptor (DTR) renders murine cells susceptible to diphtheria toxin (DT)-induced cell death and, thus, enables inducible target cell depletion (**Figure 2B**) (82, 108). In this sense mice in which DTR expression is controlled by the elements of the CD11c promoter have been widely used to characterize the *in vivo* functions of DCs (28, 109–112). In part through analyzing such reporter mice, however, it has become evident that CD11c expression is not entirely restricted to DCs. It is also expressed on alveolar MØs, $Ly6C^{low}$ as well as activated monocytes, plasmablasts, NK cells, and some T cells (25–29, 113). In addition, CD11c-driven fate reporter expression varies depending on the specific promoter elements used for transgenesis. CD11c.DTR mice, which were generated by conventional transgenesis using a 5.5-kb promoter element of the *Itgax* gene (109, 114), efficiently deplete most CD11c-expressing cDCs, LCs, alveolar, splenic marginal zone, and metallophilic MØs, as well as plasmablasts and T cells (27, 109, 115). However, DT-induced cell depletion in these mice is incomplete and spares certain cell types that transcribe their endogenous *Itgax* allele, including pDCs and NK cells (82, 115). Additionally, prolonged cell depletion using CD11c.DTR mice requires the use of bone marrow chimeras, possibly because of aberrant DTR expression on non-immune cells (82, 108, 112). Notably, this is not the case in CD11c.DOG and CD11c.LuciDTR mice, which were generated using bacterial artificial chromosome (BAC) transgenesis to place DTR under control of the extended regulatory region of the *Itgax* gene and in which DTR expression seems to more faithfully represent endogenous CD11c expression (28, 112, 115, 116). In all models, the occurrence of systemic neutrophilia and monocytosis following $CD11c^+$ cell depletion (28, 115, 117) adds another layer of complexity to deciphering the cellular function and lineage affiliation of DCs.

The realization that CD11c is not restricted to DCs in all instances nurtured the search for more specific lineage-defining markers. Two groups simultaneously identified the transcription factor Zbtb46 (zDC, Btbd4) as ideal candidate to distinguish cDCs, as it is expressed in pre-DCs and cDCs but not in pDCs or their precursors (37, 38). Consistently, $CD8\alpha^+$ and $CD11b^+$ cDCs in lymphoid organs as well as $CD103^+$ cDCs in non-lymphoid organs uniformly express Zbtb46 as assessed in Zbtb46-GFP (37) and Zbtb46-DTR (38) reporter mice generated by site-directed mutagenesis. In contrast, $CD11c^+MHCII^+CD11b^+$ cells in non-lymphoid organs, including lung, small intestine, and kidney, exhibit partial Zbtb46 expression (37, 38) indicating that they represent a heterogeneous population. This is consistent with reports demonstrating that these cells are of mixed monocyte and pre-DC origin (63, 91, 92, 95). Subsequently, Zbtb46 reporter mice have been used to help establish lineage relationships in a variety of tissues including

heart, pancreas, tumors, and thymus (118–121). The fact that Zbtb46 expression is also found in human DCs suggests that it may also help to identify DCs across species (48, 122).

Nevertheless, the use of Zbtb46 as lineage-defining marker requires a note of caution. Zbtb46 expression is downregulated after DC stimulation and it is found in some non-immune cells (37, 123). Despite its prominent expression in the cDC lineage, Zbtb46 appears largely dispensable for cDC development (37, 123). Instead, it may reinforce DC-specific transcriptional programs (37) and/or suppress DC activation (123). Interestingly, monocytes activated in the presence of GM-CSF ± IL-4 uniformly induce Zbtb46 expression, whereas monocyte-derived Tip-DCs that are generated following infection with *Listeria monocytogenes* do not (37). This raises the possibility that Zbtb46 may control DC-like features of monocyte-derived cells in some inflammatory situations and it will be interesting to determine if Zbtb46 controls transcriptional programs in monocytes. These data also highlight that despite its selective expression on cDC progenitors and their descendants, Zbtb46 is not necessarily an indicator of cell ontogeny.

Identifying Common Developmental Requirements

Establishing that the development and/or delineation of a cell type depends on a certain transcription or growth factor constitutes a powerful way of fate mapping that has extensively been applied to MPs (42, 51, 63, 124–141). We can now clearly delineate DCs into distinct subpopulations based on the transcriptional programs that govern their development. pDCs are distinguished from two subsets of cDCs by their dependence on E2-2 (67, 142). The differentiation of pre-DCs into $CD8\alpha^+$ cDCs in lymphoid organs and $CD103^+CD11b^-$ cDCs in non-lymphoid tissues is controlled by a set of transcription factors, including Irf8, Nfil-3, Id2, and Batf3 (124–128). Therefore, $CD8\alpha^+$ cDCs and $CD103^+$ cDCs represent a developmentally related lineage of cDCs (6, 7). Notably, these cells also exhibit a degree of functional relatedness that is, for instance, exemplified by their superior capacity to activate $CD8^+$ T cells (124, 143–145). In contrast, the development of $CD11b^+$ cDCs from pre-DCs is controlled by distinct transcription factors, including RelB, RbpJ, PU.1, and Irf4 (42, 129–136). Notably, expression of CD24 separates pre-DCs into cells that preferentially generate either $CD8\alpha^+$ or $CD11b^+$ cDCs in spleen (60) suggesting a stepwise differentiation of pre-DCs into cDCs. It will be interesting to determine whether such heterogeneity of pre-DCs also exists in the bone marrow. Notably, the extent of transcription factor dependence is linked to the genetic background of the particular mouse strain analyzed (146–148), indicating that transcriptional requirements are not always absolute or redundant factors exist (148). Consistently, $CD8\alpha^+$ DCs can develop in the absence of Batf3, Id2, and Nfil-3 (149). The local microenvironment may also contribute to shaping the diversity of the DC compartment, as in some tissues, such as the spleen and intestinal system, $CD11b^+$ cDCs can be divided into ontogenetically and functionally distinct subpopulations (36, 42, 91, 95, 131). Importantly, some of the transcription factors controlling DC differentiation in mice have also been implicated in the development of human DCs (67, 69, 71) and putative equivalent DC subpopulations exist in rat, chicken, sheep, and pig (150–153), highlighting that DC populations are conserved across species.

While several growth factors have been linked to DC differentiation, the development of all DC subsets is strongly dependent on FLT3 ligand (FLT3L) and downstream signaling events (7, 18, 154). FLT3L administration potently expands pDCs and cDCs in mice and humans (72, 73, 85, 155–157). *In vitro,* FLT3L promotes the differentiation of bone marrow progenitors from mice, humans, and pigs into functional subsets of DCs (66, 158, 159). Mice lacking FLT3L display a severe deficiency in DCs, which is also apparent, although to a lesser extent, in mice lacking its receptor CD135 or mice treated with CD135 inhibitors (63, 137, 160, 161). In contrast, FLT3L appears largely dispensable for monocyte and MØ development (137) and, therefore, FLT3L dependency is often used delineate DCs *in vivo* (18, 65, 162). The interpretation of fate mapping using mice deficient in CD135 or its ligand is however complicated by the fact that these animals also exhibit abnormalities in other hematopoietic lineages, including B, T, and NK cells (137, 163) and show evidence of systemic neutrophilia and monocytosis, as has been reported in other DC-deficient models (112, 117).

Despite the prominent expression of CD135 on DC progenitors it remains to be clarified exactly at what stage of cellular differentiation FLT3L impacts on DC development. Consistent with a role for FLT3L early in development, a reduction of bone marrow CDPs in FLT3L deficient animals has been reported but ranges from a mere twofold decrease (164) to near complete absence (78). In contrast, the numbers of MDPs and splenic pre-DCs appear largely unaffected by CD135 deficiency (85). The observation that pre-DC frequencies in non-lymphoid organs of FLT3L-deficient mice are reduced (63) and that transfer of DCs into a FLT3L-deficient environment decreases their homeostatic proliferation (85) indicates a role for FLT3L in the peripheral expansion of DCs rather than their differentiation. This interpretation would equally be consistent with the observation that DCs that develop in the absence of FLT3L are functional (137). In light of this finding it will be interesting to determine, to what extent FLT3L impacts on the development and functional regulation of other MPs. Addition of FLT3L to purified human monocytes cultured with GM-CSF ± IL-4 increases their T cell stimulatory capacity (165), although it is not clear whether this is also the case for murine monocytes. Culture of murine bone marrow with GM-CSF and IL-4 presumably mimics monocyte differentiation under the same conditions (166). When FLT3 signaling is inhibited in such bulk cultures the T cell stimulatory capacity of the output cells is reduced (161). Therefore, these data raise the possibility that FLT3L might influence monocyte differentiation into cells with functional properties of DCs also in the murine system, although a direct causality remains to be demonstrated. Further, comparative gene expression profiling revealed that upon migration to lymph nodes LCs induce CD135 expression (167), indicating that they might be capable of responding to FLT3L. Therefore, it is conceivable that FLT3L may control certain functional aspects generally associated with DCs, such as antigen presentation, in ontogenetically distinct MP subtypes, which will be interesting to formally address in the context of FLT3L or CD135 deficiency.

Dendritic cell progenitors also express CD115, the receptor for MØ colony-stimulating factor (M-CSF) (56–58, 61, 86). However, compared to the dominant role of FLT3L in DC differentiation, M-CSF-deficiency only mildly impacts on DC development (168). M-CSF deficient osteopetrotic (op/op) mice exhibit a two- to threefold reduction in splenic cDCs and pDCs, respectively, but the remaining DCs are capable of stimulating a mixed lymphocyte reaction and induce costimulatory molecules upon activation, thus appear functional (168). In contrast, M-CSF is strongly required for monocyte and MØ development (141, 169). Therefore, the observation that mice lacking CD115 exhibit reduced frequencies of $CD11c^+MHCII^+CD11b^+$ cells in non-lymphoid organs (63, 91) likely reflects the ontogenetic heterogeneity of this population (63, 91–95). Consistently, M-CSF is also required for the generation of monocyte-derived cells with features of DCs during inflammation (90). Nonetheless, M-CSF may play a role in DC development. It can promote DC differentiation *in vitro* and *in vivo* even in the absence of FLT3L, although DCs generated by M-CSF alone phenotypically and functionally differ from those induced by FLT3L (170). M-CSF-induced DC poeisis is also more efficient in FLT3L-sufficient conditions (170). *In vivo*, antibody-mediated blockade of M-CSF in pregnant mice reduces pre-DC extravasation, translating into a reduction of $CD11b^+$ DCs in the pregnant uterus (171). Whether M-CSF affects pre-DC migration also in other tissues and whether it acts in a cell intrinsic manner or by promoting the production of chemotactic factors by other cells remains to be determined (171).

In purified monocytes, GM-CSF induces phenotypic and functional attributes of DCs (88, 89, 172). Similarly, purified $CD115^+$ MDPs respond to GM-CSF by differentiating into $CD11c^+MHCII^+$ DCs (85) and GM-CSF deficiency leads to a slight reduction of bone marrow MDPs and CDPs (164). However, GM-CSF is dispensable for the differentiation of lymphoid tissue DCs (85, 173) and, therefore, it seemed likely that GM-CSF would selectively regulate the differentiation of monocytes into cells resembling DCs (23). This speculation also lead to the hypothesis that monocytes cultured in the presence of GM-CSF represent the counterpart of mo-DCs generated under conditions of inflammation/infection *in vivo* (23). Surprisingly, GM-CSF does not appear to control monocyte differentiation *in vivo* (90) and thus, GM-CSF elicited monocyte-derived cells are unlikely to be fully equivalent to inflammatory monocyte-derived cells. Rather, GM-CSF influences the homeostasis of cDCs in a variety, but not all, non-lymphoid tissues, most likely by promoting cell survival (90). Importantly, GM-CSF deficiency leads to a greater reduction of $CD103^+$ cDCs than of $CD11b^+$ cDCs (90). However, the extent of cDC reduction in the absence of GM-CSF apparently relates to the markers used for cell identification (90, 147, 164). This is most likely because GM-CSF regulates certain phenotypic as well as functional features of DCs, such as CD103 expression (174) or their ability to cross-present antigen (90, 174, 175). Therefore, the above-mentioned growth factors not only influence lineage decisions but also impact on the functional regulation of DCs, monocytes, and MØs. Elucidating the exact roles of FLT3L, GM-CSF, and M-CSF in each cell type will help to decipher the functional heterogeneity of MPs.

Cellular Barcoding

The biggest challenge for fate mapping is to trace the developmental plasticity of individual cells. This can now be achieved using "cellular barcoding," in which progenitors are tagged *in vitro* with semi-random, non-coding DNA sequences by transduction using retro- or lentiviral vectors (**Figure 2C**) (81). Therefore, the barcodes are heritable and by choosing conditions of low transduction efficiency one can ensure that each cell receives only a single barcode. Subsequently, barcode-labeled progenitors are adoptively transferred in numbers low enough to minimize the chance that two identically barcoded cells are transferred into the same recipient (**Figure 2C**). After differentiation *in vivo*, cell progeny are analyzed for their barcode repertoire using deep sequencing or custom microarray. Since each barcode represents an individual progenitor, the presence of the same barcode in more than one cell type indicates that they were generated from a single precursor (multi-potent or bi-potent, **Figure 2C**). On the other hand, if a barcode is only found in one cell type, the progenitor generated only a single cell lineage (mono-potent, **Figure 2C**) (81).

During maturation, HSCs are thought to progressively lose their self-renewal ability and become increasingly limited in their differentiation potential, ultimately giving rise to lineage-restricted progenitors (55, 176). Lymphoid primed multi-potent progenitors (LMPPs) are developmental intermediates downstream of HSCs that can give rise to various, but not all, cell lineages and are thus considered multi-potent (55, 176). Surprisingly, in barcoding experiments only a minority (3%) of single LMPPs exhibits true multi-potency, defined as the ability to generate all of the following cell lineages: B cells, DCs, and myeloid cells (monocytes and neutrophils) (49). Rather, single LMPPs differ drastically in terms of their cellular output: 10% of the progenitors contribute primarily to B cells, 10% primarily to myeloid cells but about 50% of transferred LMPPs produce predominantly DCs (49). The remaining fraction of progenitors exhibits bi-potentiality to generate combinations of the examined cell lineages (49). Therefore, LMPPs are multi-potent when analyzed as a population, however single cells exhibit unexpected lineage bias that is imprinted early in development. Why the majority of LMPPs is DC-committed (49), even though DCs constitute a minority lineage compared to B cells, remains to be clarified, although it is possible that some progenitors proliferate better than others or have certain competitive advantages. A major lineage divergence toward DCs seems to occur before or at the LMPP stage, as most HSCs analyzed by the same method are multi-potent, although even HSCs exhibit a degree of lineage bias (49, 177). Since CDPs might arise directly from LMPPs without additional developmental intermediates (79), these data infer that DCs diverge as a developmental lineage distinct from other myeloid cells early on (49).

This, again, questions the existence of a bi-potential MDP as central intermediate in the development of DCs and monocytes. Yet, it is noteworthy that even though DC-biased LMPPs are fivefold more frequent than bi-potent myeloid/DC LMPPs, mono-potent and bi-potent progenitors contribute equally to the final DC pool (49). Therefore, bi-potent progenitors seem to play a significant part in generating DCs, potentially because they have a

proliferative advantage. Resolving these issues will require further refinement of the technique at hand. The differentiation potential of progenitors may be influenced by cell isolation, processing or *in vitro* manipulation (80) and virus-mediated transformation might skew cell fate in an unforeseeable manner, as evidenced by the fact that barcoded LMPPs cannot generate T cells (49, 81). This also means that barcoding does not yet uncover the full potential of single progenitors. The early lineage bias of HSCs and LMPPs suggests that cell development may follow a model of graded commitment rather than proceeding in a truly stepwise manner (178). It will be interesting to determine, to what extent this process is regulated by epigenetic modification and how inflammatory processes might impact on lineage divergence. Future studies will benefit from the development of models allowing for *in vivo* barcoding of single cells but the labor-intensive quantification and analysis of barcoding experiments makes it difficult to follow populations in real time.

Genetic Lineage Tracing

Dynamic mapping of populations of distinct origin *in vivo* can be achieved using genetic lineage tracing based on *Cre-loxP* technology (**Figure 2D**) (80, 179). It relies on inducible reporter genes that are placed under the control of constitutively active promoters, such as the *Rosa26* locus. The reporter is most commonly a fluorescent protein that is preceded by a *loxP*-flanked *STOP* cassette and, therefore, its expression is induced only after Cre recombinase (Cre) mediated excision of the stop codon (**Figure 2D**). Since this form of labeling is genetic it is also heritable, meaning that any cell expressing Cre will pass on the label to all progeny, irrespective of continuous recombinase expression (**Figure 2D**). Since the promoter driving the reporter gene is constitutively active, labeling is irreversible and not affected by fluctuations in gene expression (**Figure 2D**) (80).

By crossing mice expressing Cre under the control of the *Clec9a* locus to *Rosa26-STOP-flox*-enhanced-yellow fluorescent protein (YFP) reporter mice (180), we have recently generated the first genetic model to trace the progeny of DNGR-1$^+$ CDPs and pre-DCs (36). In these mice, YFP expression is restricted to DCs but is not found in monocytes or MØs even in inflammatory conditions, as tested after intestinal inflammation or infection with *L. monocytogenes* (36). Nonetheless, certain limitations need to be taken into account. DNGR-1 is also expressed on CD8α$^+$/CD103$^+$ cDCs and to a lower extent on pDCs (36, 71, 181, 182) and, therefore, in these populations labeling is not a strict indicator of cell ontogeny. Further, labeling of CDP and pre-DC progeny in mice heterozygous for Cre is incomplete, possibly due to a delay in Cre protein synthesis and DNA recombination in rapidly cycling progenitors (36). Consistently, penetrance of the YFP label is increased in mice homozygous for Cre (36). The efficiency of lineage tracing experiments in such cases or when Cre expression is low may be improved by using alternate reporter constructs in which the *loxP* sites are positioned closer together, thus facilitating recombination (183).

Genetic lineage tracing does not require prior knowledge of which markers are expressed by the output cells and, thus, enables unbiased monitoring of cell ontogeny. Therefore, we were able to identify CDP-derived cells in cell populations previously thought to constitute monocytes/MØs based on the expression of surface markers, such as CD64 (36). CD64$^+$ CDP-derived cells do not express *Clec9a* message and are especially frequent in kidneys, although the presence of few YFP$^+$ cells in the CD64$^+$ component of lung and small intestine indicates that atypical CDP-derived cells also exist in other tissues (36). CD64$^+$ kidney DCs resemble yolk sac-derived F4/80^{hi} tissue-resident MØs, appear to lack Zbtb46 expression (37) and their affiliation as DCs or MØs has been debated (184). We, therefore, used adoptive transfer as additional method to confirm cell ontogeny. Surprisingly, neither purified DNGR-1$^+$ CDPs nor total bone marrow generated F4/80^{hi}CD64$^+$ CDP progeny in kidneys 1 week after adoptive transfer into irradiated recipients (36). Since kidney DCs reportedly have a slow turnover (185), it is possible that CDPs had insufficient opportunity to reach their renal niche and expand during short-term transfer experiments. Consistent with this notion, F4/80^{hi}CD64$^+$ kidney leukocytes were efficiently generated from bone marrow progenitors in long-term reconstitution experiments (36). Therefore, our data strongly support a CDP origin of CD64$^+$ kidney leukocytes, despite their phenotypic resemblance to monocytes or MØs (36). These data exemplify the power of lineage tracing in following cell ontogeny in an unbiased way, although it is possible that DNGR-1 is expressed on yet unidentified developmental intermediates.

Addressing this possibility might require tamoxifen-inducible Cre constructs that can be used to pulse label progenitor populations (80). In the future, combinatorial approaches, such as "split-Cre" fragments controlled by two different promoters (186) or an intersection where Cre and the inducible reporter are driven by two cell-specific promoters (187, 188) may be of benefit to generate improved models to lineage trace DCs. The identification of CDP-derived cells with attributes of monocytes/MØs exemplifies the insufficiency of phenotypic properties, such as surface markers, as means of accurate cell identification of MPs. It also raises the question why cells of distinct ontogeny but overlapping phenotype exist in the same tissue. Further elucidation of the specific functions of MPs in immunity will benefit from lineage tracing approaches that result in target cell deletion through the use of inducible DTR or DT subunit modules (82, 112, 189, 190).

Conclusion

The studies discussed above have significantly advanced our understanding of DC ontogeny but have also uncovered some uncertainties (**Figure 3**). While the bone marrow origin of DCs and monocytes is undisputed, the exact developmental intermediates and branching points between HSCs and DC progenitors remain to be clarified. Current data indicate that lineage imprinting toward DCs and monocytes may occur as early as LMPPs, potentially through epigenetic modification (**Figure 3**). This realization constitutes a major conceptual shift as it puts in question the existence of a bi-potential MDP and the resulting relatedness of DCs and monocytes. A definitive resolution of this question requires increasingly refined methods to genetically trace single progenitors or select DC and monocyte lineages. Nonetheless, it is clear that cDCs, pDCs, and monocytes can be separated based on their descendance from committed

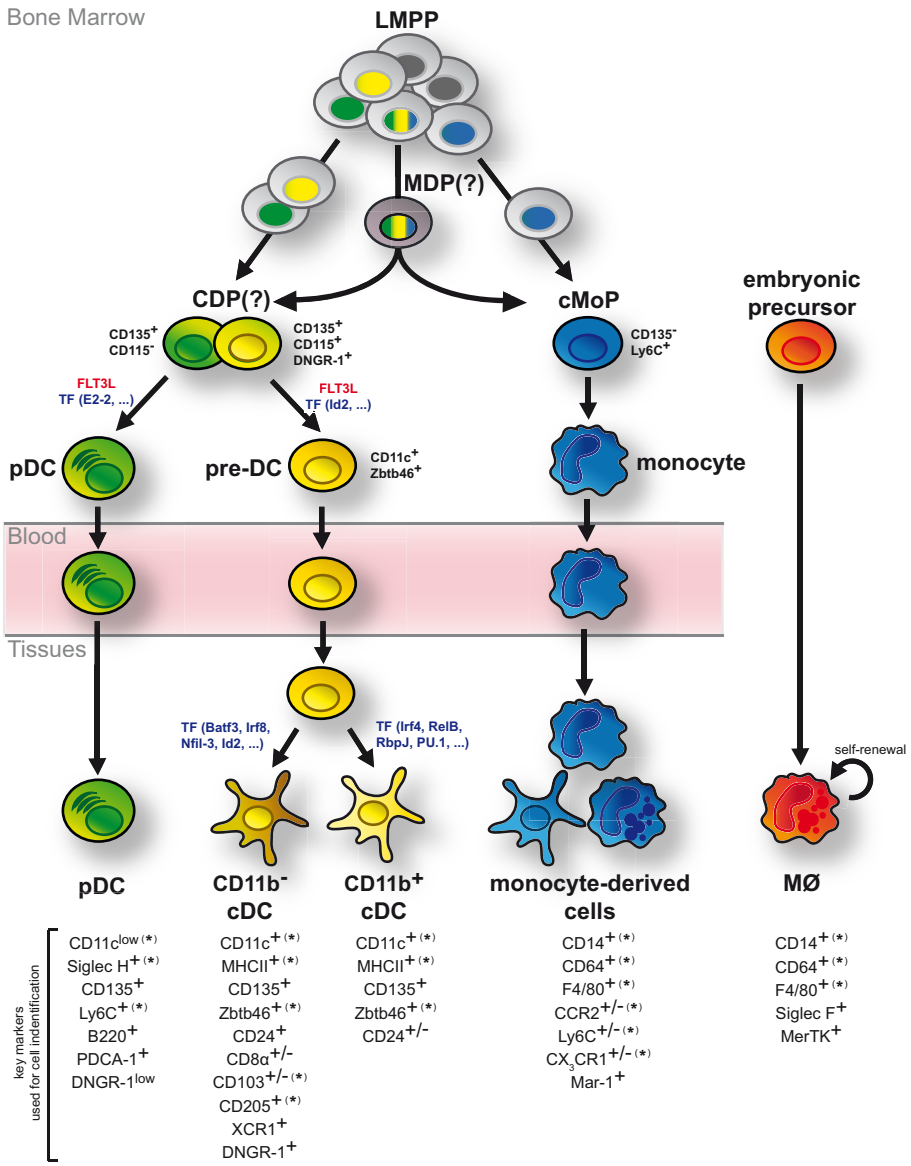

FIGURE 3 | DCs develop as independent cell lineage. Although bone marrow LMPPs are generally considered multi-potent, single LMPPs exhibit a degree of lineage bias toward generating exclusively DCs or monocytes. Such mono-potent LMPPs (single colored nuclei) may generate DC progenitors and monocyte progenitors (cMoPs) directly without additional developmental intermediates. In contrast, multi-potent LMPPs (tri-colored nuclei) presumably give rise to DCs and monocytes via bi-potent MDPs and CDPs. CDPs separate into pDC- and cDC-biased DC progenitors and can be delineated from cMoPs based on the expression of surface markers, including CD135, DNGR-1, Ly6C, and CD115 (as indicated in black). While pDCs fully develop in the bone marrow, cDCs arise via pre-DCs, which migrate through the blood stream to lymphoid and non-lymphoid tissues, where they terminally differentiate into distinct cDC subsets. These developmental processes are strongly driven by FLT3L and controlled by several transcription factors (TF; indicated in blue). Monocytes also fully differentiate in the bone marrow but upon entry into lymphoid and non-lymphoid tissues and directed by environmental cues they can acquire features of DCs or MØs. In contrast to DCs and monocytes, most tissue-resident MØs arise from embryonic progenitors and are maintained by self-renewal. Markers commonly used to distinguish pDCs, cDCs, monocyte-derived cells, and MØs in mice are shown. (*) indicates that the specified marker can be expressed on ontogenetically distinct MP subtypes in some instances. (+/−) indicates markers with heterogeneous or tissue-dependent expression on the specified MP sublineage.

developmental intermediates (**Figure 3**). Their differentiation is further driven by unique factors indicating that their developmental paths are distinct (**Figure 3**). In stark contrast to pDCs, cDCs, and monocytes, most tissue MØs arise from embryonic progenitors and are predominantly maintained by self-renewal into adulthood (**Figure 3**).

Taken together, these data unequivocally establish that DCs, monocytes, and MØs develop as unique cellular entities and although one could argue that most of this knowledge is derived from mouse studies, developmental parallels have been observed in other species (66–73). Despite these advances, we are at a loss for a universal definition of DCs that is readily accessible to

experts within and outside the field of MP biology. In light of this recognition, it has been suggested to revise the current nomenclature of MPs into a system that takes cell ontogeny into account when defining subpopulations (6). Such system would greatly aid our understanding of phagocyte biology as it remains uncertain to what extent the cellular origin of DCs, monocytes, and MØs determines the unique functionality of these cells in immunity and/or tissue homeostasis. While global profiling has revealed a role for the local tissue microenvironment in shaping the transcriptional landscape of DCs, monocytes, and MØs from different organs, certain gene signatures and transcriptional features are set by ontogeny (167, 191–193). Therefore, the full functional diversity of DCs, monocytes, and MØs is likely shaped by both nature (ontogeny) and nurture (the environment). Since ontogeny is immutable it provides a more robust common denominator for cell definition that enables deciphering cellular functions without assuming preconceived functional or phenotypic relationships. DC classification based on cell ancestry is a work in progress but its implementation will ultimately yield a more robust and transparent way of cell definition.

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References

1. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* (1973) **137**(5):1142–62. doi:10.1084/jem.137.5.1142
2. Steinman RM, Witmer MD. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc Natl Acad Sci U S A* (1978) **75**(10):5132–6. doi:10.1073/pnas.75.10.5132
3. Steinman RM, Idoyaga J. Features of the dendritic cell lineage. *Immunol Rev* (2010) **234**(1):5–17. doi:10.1111/j.0105-2896.2009.00888.x
4. van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. *J Exp Med* (1968) **128**(3):415–35. doi:10.1084/jem.128.3.415
5. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ* (1972) **46**(6):845–52.
6. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**(8):571–8. doi:10.1038/nri3712
7. Hashimoto D, Miller J, Merad M. Dendritic cell and macrophage heterogeneity in vivo. *Immunity* (2011) **35**(3):323–35. doi:10.1016/j.immuni.2011.09.007
8. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* (2005) **5**(12):953–64. doi:10.1038/nri1733
9. van Furth R. Identification of mononuclear phagocytes: overview and definitions. In: Adams DO, Edelson PJ, Koren H, editors. *Methods for Studying Mononuclear Phagocytes*. New York: Academic Press (1980). p. 243–52.
10. Schraml BU, Reis e Sousa C. Defining dendritic cells. *Curr Opin Immunol* (2015) **32**:13–20. doi:10.1016/j.coi.2014.11.001
11. Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ. Unravelling mononuclear phagocyte heterogeneity. *Nat Rev Immunol* (2010) **10**(6):453–60. doi:10.1038/nri2784
12. Hume DA. Macrophages as APC and the dendritic cell myth. *J Immunol* (2008) **181**(9):5829–35. doi:10.4049/jimmunol.181.9.5829
13. Hume DA. The mononuclear phagocyte system. *Curr Opin Immunol* (2006) **18**(1):49–53. doi:10.1016/j.coi.2005.11.008
14. Hume DA, Ross IL, Himes SR, Sasmono RT, Wells CA, Ravasi T. The mononuclear phagocyte system revisited. *J Leukoc Biol* (2002) **72**(4):621–7.
15. Reizis B. Regulation of plasmacytoid dendritic cell development. *Curr Opin Immunol* (2010) **22**(2):206–11. doi:10.1016/j.coi.2010.01.005
16. Heath WR, Carbone FR. Dendritic cell subsets in primary and secondary T cell responses at body surfaces. *Nat Immunol* (2009) **10**(12):1237–44. doi:10.1038/ni.1822
17. Briseño CG, Murphy TL, Murphy KM. Complementary diversification of dendritic cells and innate lymphoid cells. *Curr Opin Immunol* (2014) **29C**:69–78. doi:10.1016/j.coi.2014.04.006
18. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* (2013) **31**:563–604. doi:10.1146/annurev-immunol-020711-074950
19. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science* (2010) **327**(5966):656–61. doi:10.1126/science.1178331
20. Nussenzweig MC, Steinman RM, Witmer MD, Gutchinov B. A monoclonal antibody specific for mouse dendritic cells. *Proc Natl Acad Sci U S A* (1982) **79**(1):161–5. doi:10.1073/pnas.79.1.161
21. Steinman RM, Kaplan G, Witmer MD, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *J Exp Med* (1979) **149**(1):1–16. doi:10.1084/jem.149.1.1
22. Metlay JP, Witmer-Pack MD, Agger R, Crowley MT, Lawless D, Steinman RM. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. *J Exp Med* (1990) **171**(5):1753–71. doi:10.1084/jem.171.5.1753
23. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* (2007) **7**(1):19–30. doi:10.1038/nri1996
24. Mildner A, Yona S, Jung S. A close encounter of the third kind: monocyte-derived cells. *Adv Immunol* (2013) **120**:69–103. doi:10.1016/B978-0-12-417028-5.00003-X
25. Carlens J, Wahl B, Ballmaier M, Bulfone-Paus S, Forster R, Pabst O. Common gamma-chain-dependent signals confer selective survival of eosinophils in the murine small intestine. *J Immunol* (2009) **183**(9):5600–7. doi:10.4049/jimmunol.0801581
26. Vermaelen K, Pauwels R. Accurate and simple discrimination of mouse pulmonary dendritic cell and macrophage populations by flow cytometry: methodology and new insights. *Cytometry A* (2004) **61**(2):170–7. doi:10.1002/cyto.a.20064
27. Probst HC, Tschannen K, Odermatt B, Schwendener R, Zinkernagel RM, Van Den Broek M. Histological analysis of CD11c-DTR/GFP mice after in vivo depletion of dendritic cells. *Clin Exp Immunol* (2005) **141**(3):398–404. doi:10.1111/j.1365-2249.2005.02868.x
28. Hochweller K, Striegler J, Hämmerling GJ, Garbi N. A novel CD11c.DTR transgenic mouse for depletion of dendritic cells reveals their requirement for homeostatic proliferation of natural killer cells. *Eur J Immunol* (2008) **38**(10):2776–83. doi:10.1002/eji.200838659
29. Huleatt JW, Lefrançois L. Antigen-driven induction of CD11c on intestinal intraepithelial lymphocytes and CD8+ T cells in vivo. *J Immunol* (1995) **154**(11):5684–93.
30. Rubtsov AV, Rubtsova K, Fischer A, Meehan RT, Gillis JZ, Kappler JW, et al. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c(+) B-cell population is important for the development of autoimmunity. *Blood* (2011) **118**(5):1305–15. doi:10.1182/blood-2011-01-331462
31. Drutman SB, Kendall JC, Trombetta ES. Inflammatory spleen monocytes can upregulate CD11c expression without converting into dendritic cells. *J Immunol* (2012) **188**(8):3603–10. doi:10.4049/jimmunol.1102741
32. Hebel K, Griewank K, Inamine A, Chang H-D, Müller-Hilke B, Fillatreau S, et al. Plasma cell differentiation in T-independent type 2 immune responses is independent of CD11c(high) dendritic cells. *Eur J Immunol* (2006) **36**(11):2912–9. doi:10.1002/eji.200636356

33. Kim K-W, Vallon-Eberhard A, Zigmond E, Farache J, Shezen E, Shakhar G, et al. In vivo structure/function and expression analysis of the CX3C chemokine fractalkine. *Blood* (2011) **118**(22):e156–67. doi:10.1182/blood-2011-04-348946
34. Farache J, Koren I, Milo I, Gurevich I, Kim KW, Zigmond E, et al. Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity* (2013) **38**(3):581–95. doi:10.1016/j.immuni.2013.01.009
35. Bergstresser PR, Sullivan S, Streilein JW, Tigelaar RE. Origin and function of Thy-1+ dendritic epidermal cells in mice. *J Invest Dermatol* (1985) **85**(1 Suppl):85s–90s. doi:10.1111/1523-1747.ep12275516
36. Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* (2013) **154**(4):843–58. doi:10.1016/j.cell.2013.07.014
37. Satpathy AT, Kc W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med* (2012) **209**(6):1135–52. doi:10.1084/jem.20120030
38. Meredith MM, Liu K, Darrasse-Jèze G, Kamphorst AO, Schreiber HA, Guermonprez P, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J Exp Med* (2012) **209**(6):1153–65. doi:10.1084/jem.20112675
39. Mashayekhi M, Sandau MM, Dunay IR, Frickel EM, Khan A, Goldszmid RS, et al. CD8α(+) dendritic cells are the critical source of interleukin-12 that controls acute infection by *Toxoplasma gondii* tachyzoites. *Immunity* (2011) **35**(2):249–59. doi:10.1016/j.immuni.2011.08.008
40. Reis e Sousa C, Hieny S, Scharton-Kersten T, Jankovic D, Charest H, Germain RN, et al. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J Exp Med* (1997) **186**(11):1819–29. doi:10.1084/jem.186.11.1819
41. Whitney PG, Bar E, Osorio F, Rogers NC, Schraml BU, Deddouche S, et al. Syk signaling in dendritic cells orchestrates innate resistance to systemic fungal infection. *PLoS Pathog* (2014) **10**(7):e1004276. doi:10.1371/journal.ppat.1004276
42. Satpathy AT, Briseño CG, Lee JS, Ng D, Manieri NA, Kc W, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol* (2013) **14**(9):937–48. doi:10.1038/ni.2679
43. Kinnebrew MA, Buffie CG, Diehl GE, Zenewicz LA, Leiner I, Hohl TM, et al. Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. *Immunity* (2012) **36**(2):276–87. doi:10.1016/j.immuni.2011.12.011
44. Arora P, Baena A, Yu KOA, Saini NK, Kharkwal SS, Goldberg MF, et al. A single subset of dendritic cells controls the cytokine bias of natural killer T cell responses to diverse glycolipid antigens. *Immunity* (2014) **40**(1):105–16. doi:10.1016/j.immuni.2013.12.004
45. Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, et al. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* (2013) **39**(3):599–610. doi:10.1016/j.immuni.2013.08.007
46. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* (2013) **38**(2):322–35. doi:10.1016/j.immuni.2012.10.016
47. Pozzi LA, Maciaszek JW, Rock KL. Both dendritic cells and macrophages can stimulate naive CD8 T cells in vivo to proliferate, develop effector function, and differentiate into memory cells. *J Immunol* (2005) **175**(4):2071–81. doi:10.4049/jimmunol.175.4.2071
48. McGovern N, Schlitzer A, Gunawan M, Jardine L, Shin A, Poyner E, et al. Human dermal CD14+ cells are a transient population of monocyte-derived macrophages. *Immunity* (2014) **41**(3):465–77. doi:10.1016/j.immuni.2014.08.006
49. Naik SH, Perié L, Swart E, Gerlach C, van Rooij N, de Boer RJ, et al. Diverse and heritable lineage imprinting of early haematopoietic progenitors. *Nature* (2013) **496**(7444):229–32. doi:10.1038/nature12013
50. Yona S, Kim K-W, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**(1):79–91. doi:10.1016/j.immuni.2012.12.001
51. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**(6077):86–90. doi:10.1126/science.1219179
52. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko A-C, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* (2013) **14**(8):821–30. doi:10.1038/ni.2638
53. Haniffa M, Ginhoux F, Wang X-N, Bigley V, Abel M, Dimmick I, et al. Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. *J Exp Med* (2009) **206**(2):371–85. doi:10.1084/jem.20081633
54. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**(4):792–804. doi:10.1016/j.immuni.2013.04.004
55. Iwasaki H, Akashi K. Myeloid lineage commitment from the hematopoietic stem cell. *Immunity* (2007) **26**(6):726–40. doi:10.1016/j.immuni.2007.06.004
56. Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, Littman DR, et al. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* (2006) **311**(5757):83–7. doi:10.1126/science.1117729
57. Naik SH, Sathe P, Park H-Y, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol* (2007) **8**(11):1217–26. doi:10.1038/ni1522
58. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, Manz MG. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol* (2007) **8**(11):1207–16. doi:10.1038/ni1518
59. Shortman K, Sathe P, Vremec D, Naik S, O'Keeffe M. Plasmacytoid dendritic cell development. *Adv Immunol* (2013) **120**:105–26. doi:10.1016/B978-0-12-417028-5.00004-1
60. Naik SH, Metcalf D, Van Nieuwenhuijze A, Wicks I, Wu L, O'Keeffe M, et al. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat Immunol* (2006) **7**(6):663–71. doi:10.1038/ni1340
61. Liu K, Victora GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, et al. In vivo analysis of dendritic cell development and homeostasis. *Science* (2009) **324**(5925):392–7. doi:10.1126/science.1170540
62. Liu K, Waskow C, Liu X, Yao K, Hoh J, Nussenzweig M. Origin of dendritic cells in peripheral lymphoid organs of mice. *Nat Immunol* (2007) **8**(6):578–83. doi:10.1038/ni1462
63. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* (2009) **206**(13):3115–30. doi:10.1084/jem.20091756
64. Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* (2003) **19**(1):59–70. doi:10.1016/S1074-7613(03)00171-7
65. Haniffa M, Collin M, Ginhoux F. Ontogeny and functional specialization of dendritic cells in human and mouse. *Adv Immunol* (2013) **120**:1–49. doi:10.1016/B978-0-12-417028-5.00001-6
66. Guzylack-Piriou L, Alves MP, McCullough KC, Summerfield A. Porcine Flt3 ligand and its receptor: generation of dendritic cells and identification of a new marker for porcine dendritic cells. *Dev Comp Immunol* (2010) **34**(4):455–64. doi:10.1016/j.dci.2009.12.006
67. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* (2008) **135**(1):37–48. doi:10.1016/j.cell.2008.09.016
68. Maraskovsky E, Daro E, Roux E, Teepe M, Maliszewski CR, Hoek J, et al. In vivo generation of human dendritic cell subsets by Flt3 ligand. *Blood* (2000) **96**(3):878–84.
69. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. IRF8 mutations and human dendritic-cell immunodeficiency. *N Engl J Med* (2011) **365**(2):127–38. doi:10.1056/NEJMoa1100066
70. Harada S, Kimura T, Fujiki H, Nakagawa H, Ueda Y, Itoh T, et al. Flt3 ligand promotes myeloid dendritic cell differentiation of human hematopoietic progenitor cells: possible application for cancer immunotherapy. *Int J Oncol* (2007) **30**(6):1461–8. doi:10.3892/ijo.30.6.1461

71. Poulin LF, Reyal Y, Uronen-Hansson H, Schraml BU, Sancho D, Murphy KM, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. *Blood* (2012) **119**(25):6052–62. doi:10.1182/blood-2012-01-406967
72. Breton G, Lee J, Zhou YJ, Schreiber JJ, Keler T, Puhr S, et al. Circulating precursors of human CD1c+ and CD141+ dendritic cells. *J Exp Med* (2015) **212**(3):401–13. doi:10.1084/jem.20141441
73. Lee J, Breton G, Oliveira TYK, Zhou YJ, Aljoufi A, Puhr S, et al. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. *J Exp Med* (2015) **212**(3):385–99. doi:10.1084/jem.20141442
74. Traver D, Akashi K, Manz M, Merad M, Miyamoto T, Engleman EG, et al. Development of CD8alpha-positive dendritic cells from a common myeloid progenitor. *Science* (2000) **290**(5499):2152–4. doi:10.1126/science.290.5499.2152
75. Schlenner SM, Madan V, Busch K, Tietz A, Läufle C, Costa C, et al. Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus. *Immunity* (2010) **32**(3):426–36. doi:10.1016/j.immuni.2010.03.005
76. Luche H, Ardouin L, Teo P, See P, Henri S, Merad M, et al. The earliest intrathymic precursors of CD8α(+) thymic dendritic cells correspond to myeloid-type double-negative 1c cells. *Eur J Immunol* (2011) **41**(8):2165–75. doi:10.1002/eji.201141728
77. Sathe P, Vremec D, Wu L, Corcoran L, Shortman K. Convergent differentiation: myeloid and lymphoid pathways to murine plasmacytoid dendritic cells. *Blood* (2013) **121**(1):11–9. doi:10.1182/blood-2012-02-413336
78. Sathe P, Metcalf D, Vremec D, Naik SH, Langdon WY, Huntington ND, et al. Lymphoid tissue and plasmacytoid dendritic cells and macrophages do not share a common macrophage-dendritic cell-restricted progenitor. *Immunity* (2014) **41**(1):104–15. doi:10.1016/j.immuni.2014.05.020
79. Onai N, Kurabayashi K, Hosoi-Amaike M, Toyama-Sorimachi N, Matsushima K, Inaba K, et al. A clonogenic progenitor with prominent plasmacytoid dendritic cell developmental potential. *Immunity* (2013) **38**(5):943–57. doi:10.1016/j.immuni.2013.04.006
80. Kretzschmar K, Watt FM. Lineage tracing. *Cell* (2012) **148**(1–2):33–45. doi:10.1016/j.cell.2012.01.002
81. Naik SH, Schumacher TN, Perié L. Cellular barcoding: a technical appraisal. *Exp Hematol* (2014) **42**(8):598–608. doi:10.1016/j.exphem.2014.05.003
82. Bar-On L, Jung S. Defining dendritic cells by conditional and constitutive cell ablation. *Immunol Rev* (2010) **234**(1):76–89. doi:10.1111/j.0105-2896.2009.00875.x
83. Plett PA, Frankovitz SM, Orschell-Traycoff CM. In vivo trafficking, cell cycle activity, and engraftment potential of phenotypically defined primitive hematopoietic cells after transplantation into irradiated or nonirradiated recipients. *Blood* (2002) **100**(10):3545–52. doi:10.1182/blood.V100.10.3545
84. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* (2007) **204**(1):171–80. doi:10.1084/jem.20061011
85. Waskow C, Liu K, Darrasse-Jèze G, Guermonprez P, Ginhoux F, Merad M, et al. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol* (2008) **9**(6):676–83. doi:10.1038/ni.1615
86. Auffray C, Fogg DK, Narni-Mancinelli E, Senechal B, Trouillet C, Saederup N, et al. CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. *J Exp Med* (2009) **206**(3):595–606. doi:10.1084/jem.20081385
87. Schlitzer A, Loschko J, Mair K, Vogelmann R, Henkel L, Einwächter H, et al. Identification of CCR9- murine plasmacytoid DC precursors with plasticity to differentiate into conventional DCs. *Blood* (2011) **117**(24):6562–70. doi:10.1182/blood-2010-12-326678
88. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* (1994) **179**(4):1109–18. doi:10.1084/jem.179.4.1109
89. Romani N, Gruner S, Brang D, Kämpgen E, Lenz A, Trockenbacher B, et al. Proliferating dendritic cell progenitors in human blood. *J Exp Med* (1994) **180**(1):83–93. doi:10.1084/jem.180.1.83
90. Greter M, Helft J, Chow A, Hashimoto D, Mortha A, Agudo-Cantero J, et al. GM-CSF controls nonlymphoid tissue dendritic cell homeostasis but is dispensable for the differentiation of inflammatory dendritic cells. *Immunity* (2012) **36**(6):1031–46. doi:10.1016/j.immuni.2012.03.027
91. Bogunovic M, Ginhoux F, Helft J, Shang L, Hashimoto D, Greter M, et al. Origin of the lamina propria dendritic cell network. *Immunity* (2009) **31**(3):513–25. doi:10.1016/j.immuni.2009.08.010
92. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity* (2009) **31**(3):502–12. doi:10.1016/j.immuni.2009.06.025
93. Tamoutounour S, Henri S, Lelouard H, de Bovis B, de Haar C, van der Woude CJ, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol* (2012) **42**(12):3150–66. doi:10.1002/eji.201242847
94. Langlet C, Tamoutounour S, Henri S, Luche H, Ardouin L, Grégoire C, et al. CD64 expression distinguishes monocyte-derived and conventional dendritic cells and reveals their distinct role during intramuscular immunization. *J Immunol* (2012) **188**(4):1751–60. doi:10.4049/jimmunol.1102744
95. Scott CL, Bain CC, Wright PB, Sichien D, Kotarsky K, Persson EK, et al. CCR2(+)CD103(-) intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunol* (2014) **8**(2):327–39. doi:10.1038/mi.2014.70
96. Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, et al. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol* (2002) **3**(12):1135–41. doi:10.1038/ni852
97. Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai X-M, et al. Langerhans cells arise from monocytes in vivo. *Nat Immunol* (2006) **7**(3):265–73. doi:10.1038/ni1307
98. Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat Rev Immunol* (2008) **8**(12):935–47. doi:10.1038/nri2455
99. Romani N, Clausen BE, Stoitzner P. Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. *Immunol Rev* (2010) **234**(1):120–41. doi:10.1111/j.0105-2896.2009.00886.x
100. Ginhoux F, Merad M. Ontogeny and homeostasis of Langerhans cells. *Immunol Cell Biol* (2010) **88**(4):387–92. doi:10.1038/icb.2010.38
101. Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med* (2012) **209**(6):1167–81. doi:10.1084/jem.20120340
102. Chorro L, Sarde A, Li M, Woollard KJ, Chambon P, Malissen B, et al. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. *J Exp Med* (2009) **206**(13):3089–100. doi:10.1084/jem.20091586
103. Hume DA. Applications of myeloid-specific promoters in transgenic mice support in vivo imaging and functional genomics but do not support the concept of distinct macrophage and dendritic cell lineages or roles in immunity. *J Leukoc Biol* (2011) **89**(4):525–38. doi:10.1189/jlb.0810472
104. Lindquist RL, Shakhar G, Dudziak D, Wardemann H, Eisenreich T, Dustin ML, et al. Visualizing dendritic cell networks in vivo. *Nat Immunol* (2004) **5**(12):1243–50. doi:10.1038/ni1139
105. Thornton EE, Looney MR, Bose O, Sen D, Sheppard D, Locksley R, et al. Spatiotemporally separated antigen uptake by alveolar dendritic cells and airway presentation to T cells in the lung. *J Exp Med* (2012) **209**(6):1183–99. doi:10.1084/jem.20112667
106. Choi J-H, Do Y, Cheong C, Koh H, Boscardin SB, Oh Y-S, et al. Identification of antigen-presenting dendritic cells in mouse aorta and cardiac valves. *J Exp Med* (2009) **206**(3):497–505. doi:10.1084/jem.20082129
107. Khanna KM, Blair DA, Vella AT, McSorley SJ, Datta SK, Lefrançois L. T cell and APC dynamics in situ control the outcome of vaccination. *J Immunol* (2010) **185**(1):239–52. doi:10.4049/jimmunol.0901047
108. Bennett CL, Clausen BE. DC ablation in mice: promises, pitfalls, and challenges. *Trends Immunol* (2007) **28**(12):525–31. doi:10.1016/j.it.2007.08.011
109. Jung S, Unutmaz D, Wong P, Sano G-I, de los Santos K, Sparwasser T, et al. In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. *Immunity* (2002) **17**(2):211–20. doi:10.1016/S1074-7613(02)00365-5
110. Tittel AP, Heuser C, Ohliger C, Knolle PA, Engel DR, Kurts C. Kidney dendritic cells induce innate immunity against bacterial pyelonephritis. *J Am Soc Nephrol* (2011) **22**(8):1435–41. doi:10.1681/ASN.2010101072
111. Bar-On L, Birnberg T, Kim K-W, Jung S. Dendritic cell-restricted CD80/86 deficiency results in peripheral regulatory T-cell reduction but is not associated

with lymphocyte hyperactivation. *Eur J Immunol* (2011) **41**(2):291–8. doi:10.1002/eji.201041169
112. van Blijswijk J, Schraml BU, Reis e Sousa C. Advantages and limitations of mouse models to deplete dendritic cells. *Eur J Immunol* (2013) **43**(1):22–6. doi:10.1002/eji.201243022
113. Racine R, Chatterjee M, Winslow GM. CD11c expression identifies a population of extrafollicular antigen-specific splenic plasmablasts responsible for CD4 T-independent antibody responses during intracellular bacterial infection. *J Immunol* (2008) **181**(2):1375–85. doi:10.4049/jimmunol.181.2.1375
114. Brocker T, Riedinger M, Karjalainen K. Driving gene expression specifically in dendritic cells. *Adv Exp Med Biol* (1997) **417**:55–7. doi:10.1007/978-1-4757-9966-8_9
115. Tittel AP, Heuser C, Ohliger C, Llanto C, Yona S, Hämmerling GJ, et al. Functionally relevant neutrophilia in CD11c diphtheria toxin receptor transgenic mice. *Nat Methods* (2012) **9**(4):385–90. doi:10.1038/nmeth.1905
116. Bar-On L, Jung S. Defining in vivo dendritic cell functions using CD11c-DTR transgenic mice. *Methods Mol Biol* (2010) **595**:429–42. doi:10.1007/978-1-60761-421-0_28
117. Jiao J, Dragomir A-C, Kocabayoglu P, Rahman AH, Chow A, Hashimoto D, et al. Central role of conventional dendritic cells in regulation of bone marrow release and survival of neutrophils. *J Immunol* (2014) **192**(7):3374–82. doi:10.4049/jimmunol.1300237
118. Gardner JM, Metzger TC, McMahon EJ, Au-Yeung BB, Krawisz AK, Lu W, et al. Extrathymic aire-expressing cells are a distinct bone marrow-derived population that induce functional inactivation of CD4+ T Cells. *Immunity* (2013) **39**(3):560–72. doi:10.1016/j.immuni.2013.08.005
119. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* (2014) **40**(1):91–104. doi:10.1016/j.immuni.2013.11.019
120. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell* (2014) **26**(5):638–52. doi:10.1016/j.ccell.2014.09.007
121. Ferris ST, Carrero JA, Mohan JF, Calderon B, Murphy KM, Unanue ER. A minor subset of Batf3-dependent antigen-presenting cells in islets of langerhans is essential for the development of autoimmune diabetes. *Immunity* (2014) **41**(4):657–69. doi:10.1016/j.immuni.2014.09.012
122. Segura E, Touzot M, Bohineust A, Cappuccio A, Chiocchia G, Hosmalin A, et al. Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity* (2013) **38**(2):336–48. doi:10.1016/j.immuni.2012.10.018
123. Meredith MM, Liu K, Kamphorst AO, Idoyaga J, Yamane A, Guermonprez P, et al. Zinc finger transcription factor zDC is a negative regulator required to prevent activation of classical dendritic cells in the steady state. *J Exp Med* (2012) **209**(9):1583–93. doi:10.1084/jem.20121003
124. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* (2008) **322**(5904):1097–100. doi:10.1126/science.1164206
125. Edelson BT, Kc W, Juang R, Kohyama M, Benoit LA, Klekotka PA, et al. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J Exp Med* (2010) **207**(4):823–36. doi:10.1084/jem.20091627
126. Schiavoni G, Mattei F, Sestili P, Borghi P, Venditti M, Morse HC, et al. ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells. *J Exp Med* (2002) **196**(11):1415–25. doi:10.1084/jem.20021263
127. Hacker C, Kirsch RD, Ju X-S, Hieronymus T, Gust TC, Kuhl C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat Immunol* (2003) **4**(4):380–6. doi:10.1038/ni903
128. Kashiwada M, Pham NL, Pewe LL, Harty JT, Rothman PB. NFIL3/E4BP4 is a key transcription factor for CD8alpha(+) dendritic cell development. *Blood* (2011) **117**(23):6193–7. doi:10.1182/blood-2010-07-295873
129. Suzuki S, Honma K, Matsuyama T, Suzuki K, Toriyama K, Akitoyo I, et al. Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha- dendritic cell development. *Proc Natl Acad Sci U S A* (2004) **101**(24):8981–6. doi:10.1073/pnas.0402139101
130. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J Exp Med* (2007) **204**(7):1653–64. doi:10.1084/jem.20062648
131. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* (2011) **35**(5):780–91. doi:10.1016/j.immuni.2011.08.013
132. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* (2013) **38**(5):970–83. doi:10.1016/j.immuni.2013.04.011
133. Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hägerbrand K, Marsal J, et al. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* (2013) **38**(5):958–69. doi:10.1016/j.immuni.2013.03.009
134. Tamura T, Tailor P, Yamaoka K, Kong HJ, Tsujimura H, O'shea JJ, et al. IFN regulatory factor-4 and -8 govern dendritic cell subset development and their functional diversity. *J Immunol* (2005) **174**(5):2573–81. doi:10.4049/jimmunol.174.5.2573
135. Wu L, D'Amico A, Winkel KD, Suter M, Lo D, Shortman K. RelB is essential for the development of myeloid-related CD8alpha- dendritic cells but not of lymphoid-related CD8alpha+ dendritic cells. *Immunity* (1998) **9**(6):839–47. doi:10.1016/S1074-7613(00)80649-4
136. Guerriero A, Langmuir PB, Spain LM, Scott EW. PU.1 is required for myeloid-derived but not lymphoid-derived dendritic cells. *Blood* (2000) **95**(3):879–85.
137. McKenna HJ, Stocking KL, Miller RE, Brasel K, De Smedt T, Maraskovsky E, et al. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* (2000) **95**(11):3489–97.
138. Kohyama M, Ise W, Edelson BT, Wilker PR, Hildner K, Mejia C, et al. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. *Nature* (2009) **457**(7227):318–21. doi:10.1038/nature07472
139. Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat Immunol* (2012) **13**(8):753–60. doi:10.1038/ni.2360
140. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. *Immunity* (2012) **37**(6):1050–60. doi:10.1016/j.immuni.2012.11.001
141. Wiktor-Jedrzejczak WW, Ahmed A, Szczylik C, Skelly RR. Hematological characterization of congenital osteopetrosis in op/op mouse. Possible mechanism for abnormal macrophage differentiation. *J Exp Med* (1982) **156**(5):1516–27. doi:10.1084/jem.156.5.1516
142. Ghosh HS, Cisse B, Bunin A, Lewis KL, Reizis B. Continuous expression of the transcription factor e2-2 maintains the cell fate of mature plasmacytoid dendritic cells. *Immunity* (2010) **33**(6):905–16. doi:10.1016/j.immuni.2010.11.023
143. Helft J, Manicassamy B, Guermonprez P, Hashimoto D, Silvin A, Agudo J, et al. Cross-presenting CD103+ dendritic cells are protected from influenza virus infection. *J Clin Invest* (2012) **122**(11):4037–47. doi:10.1172/JCI60659
144. Desch AN, Randolph GJ, Murphy K, Gautier EL, Kedl RM, Lahoud MH, et al. CD103+ pulmonary dendritic cells preferentially acquire and present apoptotic cell-associated antigen. *J Exp Med* (2011) **208**(9):1789–97. doi:10.1084/jem.20110538
145. Bedoui S, Whitney PG, Waithman J, Eidsmo L, Wakim L, Caminschi I, et al. Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. *Nat Immunol* (2009) **10**(5):488–95. doi:10.1038/ni.1724
146. Murphy KM. Transcriptional control of dendritic cell development. *Adv Immunol* (2013) **120**:239–67. doi:10.1016/B978-0-12-417028-5.00009-0
147. Edelson BT, Bradstreet TR, Kc W, Hildner K, Herzog JW, Sim J, et al. Batf3-dependent CD11b(low/-) peripheral dendritic cells are GM-CSF-independent and are not required for Th cell priming after subcutaneous immunization. *PLoS One* (2011) **6**(10):e25660. doi:10.1371/journal.pone.0025660
148. Tussiwand R, Lee W-L, Murphy TL, Mashayekhi M, Wumesh KC, Albring JC, et al. Compensatory dendritic cell development mediated by BATF-IRF interactions. *Nature* (2012) **490**(7421):502–7. doi:10.1038/nature11531
149. Seillet C, Jackson JT, Markey KA, Brady HJM, Hill GR, MacDonald KPA, et al. CD8α+ DCs can be induced in the absence of transcription factors Id2, Nfil3, and Batf3. *Blood* (2013) **121**(9):1574–83. doi:10.1182/blood-2012-07-445650

150. Contreras V, Urien C, Guiton R, Alexandre Y, Vu Manh T-P, Andrieu T, et al. Existence of CD8α-like dendritic cells with a conserved functional specialization and a common molecular signature in distant mammalian species. *J Immunol* (2010) **185**(6):3313–25. doi:10.4049/jimmunol.1000824
151. Marquet F, Bonneau M, Pascale F, Urien C, Kang C, Schwartz-Cornil I, et al. Characterization of dendritic cells subpopulations in skin and afferent lymph in the swine model. *PLoS One* (2011) **6**(1):e16320. doi:10.1371/journal.pone.0016320
152. Vu Manh TP, Marty H, Sibille P, Le Vern Y, Kaspers B, Dalod M, et al. Existence of conventional dendritic cells in Gallus gallus revealed by comparative gene expression profiling. *J Immunol* (2014) **192**(10):4510–7. doi:10.4049/jimmunol.1303405
153. Hubert FX, Voisine C, Louvet C, Heslan M, Josien R. Rat plasmacytoid dendritic cells are an abundant subset of MHC class II+ CD4+CD11b-OX62- and type I IFN-producing cells that exhibit selective expression of toll-like receptors 7 and 9 and strong responsiveness to CpG. *J Immunol* (2004) **172**(12):7485–94. doi:10.4049/jimmunol.172.12.7485
154. Sathaliyawala T, O'Gorman WE, Greter M, Bogunovic M, Konjufca V, Hou ZE, et al. Mammalian target of rapamycin controls dendritic cell development downstream of Flt3 ligand signaling. *Immunity* (2010) **33**(4):597–606. doi:10.1016/j.immuni.2010.09.012
155. Brasel K, McKenna HJ, Morrissey PJ, Charrier K, Morris AE, Lee CC, et al. Hematologic effects of flt3 ligand in vivo in mice. *Blood* (1996) **88**(6):2004–12.
156. Maraskovsky E, Brasel K, Teepe M, Roux ER, Lyman SD, Shortman K, et al. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med* (1996) **184**(5):1953–62. doi:10.1084/jem.184.5.1953
157. Pulendran B, Banchereau J, Burkeholder S, Kraus E, Guinet E, Chalouni C, et al. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J Immunol* (2000) **165**(1):566–72. doi:10.4049/jimmunol.165.1.566
158. Naik SH, Proietto AI, Wilson NS, Dakic A, Schnorrer P, Fuchsberger M, et al. Cutting edge: generation of splenic CD8+ and CD8- dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. *J Immunol* (2005) **174**(11):6592–7. doi:10.4049/jimmunol.174.11.6592
159. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen J-L, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**(6):1261–71. doi:10.1084/jem.20092618
160. Tussiwand R, Onai N, Mazzucchelli L, Manz MG. Inhibition of natural type I IFN-producing and dendritic cell development by a small molecule receptor tyrosine kinase inhibitor with Flt3 affinity. *J Immunol* (2005) **175**(6):3674–80. doi:10.4049/jimmunol.175.6.3674
161. Whartenby KA, Calabresi PA, McCadden E, Nguyen B, Kardian D, Wang T, et al. Inhibition of FLT3 signaling targets DCs to ameliorate autoimmune disease. *Proc Natl Acad Sci U S A* (2005) **102**(46):16741–6. doi:10.1073/pnas.0506088102
162. Jenkins SJ, Hume DA. Homeostasis in the mononuclear phagocyte system. *Trends Immunol* (2014) **35**(8):358–67. doi:10.1016/j.it.2014.06.006
163. Mackarehtschian K, Hardin JD, Moore KA, Boast S, Goff SP, Lemischka IR. Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. *Immunity* (1995) **3**(1):147–61. doi:10.1016/1074-7613(95)90167-1
164. Kingston D, Schmid MA, Onai N, Obata-Onai A, Baumjohann D, Manz MG. The concerted action of GM-CSF and Flt3-ligand on in vivo dendritic cell homeostasis. *Blood* (2009) **114**(4):835–43. doi:10.1182/blood-2009-02-206318
165. Kim S-W, Choi S-M, Choo YS, Kim I-K, Song B-W, Kim H-S. Flt3 ligand induces monocyte proliferation and enhances the function of monocyte-derived dendritic cells in vitro. *J Cell Physiol* (2014). doi:10.1002/jcp.24824
166. Xu Y, Zhan Y, Lew AM, Naik SH, Kershaw MH. Differential development of murine dendritic cells by GM-CSF versus Flt3 ligand has implications for inflammation and trafficking. *J Immunol* (2007) **179**(11):7577–84. doi:10.4049/jimmunol.179.11.7577
167. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* (2012) **13**(9):888–99. doi:10.1038/ni.2370
168. MacDonald KPA, Rowe V, Bofinger HM, Thomas R, Sasmono T, Hume DA, et al. The colony-stimulating factor 1 receptor is expressed on dendritic cells during differentiation and regulates their expansion. *J Immunol* (2005) **175**(3):1399–405. doi:10.4049/jimmunol.175.3.1399
169. Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood* (2002) **99**(1):111–20. doi:10.1182/blood.V99.1.111
170. Fancke B, Suter M, Hochrein H, O'Keeffe M. M-CSF: a novel plasmacytoid and conventional dendritic cell poietin. *Blood* (2008) **111**(1):150–9. doi:10.1182/blood-2007-05-089292
171. Tagliani E, Shi C, Nancy P, Tay C-S, Pamer EG, Erlebacher A. Coordinate regulation of tissue macrophage and dendritic cell population dynamics by CSF-1. *J Exp Med* (2011) **208**(9):1901–16. doi:10.1084/jem.20110866
172. Schreurs MW, Eggert AA, de Boer AJ, Figdor CG, Adema GJ. Generation and functional characterization of mouse monocyte-derived dendritic cells. *Eur J Immunol* (1999) **29**(9):2835–41. doi:10.1002/(SICI)1521-4141(199909)29:09<2835::AID-IMMU2835>3.3.CO;2-H
173. Vremec D, Lieschke GJ, Dunn AR, Robb L, Metcalf D, Shortman K. The influence of granulocyte/macrophage colony-stimulating factor on dendritic cell levels in mouse lymphoid organs. *Eur J Immunol* (1997) **27**(1):40–4. doi:10.1002/eji.1830270107
174. Zhan Y, Carrington EM, Van Nieuwenhuijze A, Bedoui S, Seah S, Xu Y, et al. GM-CSF increases cross-presentation and CD103 expression by mouse $CD8^+$ spleen dendritic cells. *Eur J Immunol* (2011) **41**(9):2585–95. doi:10.1002/eji.201141540
175. Sathe P, Pooley J, Vremec D, Mintern J, Jin J-O, Wu L, et al. The acquisition of antigen cross-presentation function by newly formed dendritic cells. *J Immunol* (2011) **186**(9):5184–92. doi:10.4049/jimmunol.1002683
176. Luc S, Buza-Vidas N, Jacobsen SE. Biological and molecular evidence for existence of lymphoid-primed multipotent progenitors. *Ann N Y Acad Sci* (2007) **1106**:89–94. doi:10.1196/annals.1392.023
177. Verovskaya E, Broekhuis MJ, Zwart E, Ritsema M, van Os R, de Haan G, et al. Heterogeneity of young and aged murine hematopoietic stem cells revealed by quantitative clonal analysis using cellular barcoding. *Blood* (2013) **122**(4):523–32. doi:10.1182/blood-2013-01-481135
178. Paul F, Amit I. Plasticity in the transcriptional and epigenetic circuits regulating dendritic cell lineage specification and function. *Curr Opin Immunol* (2014) **30C**:1–8. doi:10.1016/j.coi.2014.04.004
179. Vorhagen S, Jackow J, Mohor SG, Tanghe G, Tanrikulu L, Skazik-Vogt C, et al. Lineage tracing mediated by cre-recombinase activity. *J Invest Dermatol* (2015) **135**(1):e28. doi:10.1038/jid.2014.472
180. Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, et al. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* (2001) **1**:4. doi:10.1186/1471-213X-1-4
181. Caminschi I, Proietto AI, Ahmet F, Kitsoulis S, Shin Teh J, Lo JCY, et al. The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. *Blood* (2008) **112**(8):3264–73. doi:10.1182/blood-2008-05-155176
182. Sancho D, Mourão-Sá D, Joffre OP, Schulz O, Rogers NC, Pennington DJ, et al. Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. *J Clin Invest* (2008) **118**(6):2098–110. doi:10.1172/JCI34584
183. Liu J, Willet SG, Bankaitis ED, Xu Y, Wright CVE, Gu G. Non-parallel recombination limits cre-loxP-based reporters as precise indicators of conditional genetic manipulation. *Genesis* (2013) **51**(6):436–42. doi:10.1002/dvg.22384
184. Nelson PJ, Rees AJ, Griffin MD, Hughes J, Kurts C, Duffield J. The renal mononuclear phagocytic system. *J Am Soc Nephrol* (2012) **23**(2):194–203. doi:10.1681/ASN.2011070680
185. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD. Antigen presentation by dendritic cells in renal lymph nodes is linked to systemic and local injury to the kidney. *Kidney Int* (2005) **68**(3):1096–108. doi:10.1111/j.1523-1755.2005.00502.x
186. Hirrlinger J, Requardt RP, Winkler U, Wilhelm F, Schulze C, Hirrlinger PG. Split-CreERT2: temporal control of DNA recombination mediated by split-Cre protein fragment complementation. *PLoS One* (2009) **4**(12):e8354. doi:10.1371/journal.pone.0008354
187. Schreiber HA, Loschko J, Karssemeijer RA, Escolano A, Meredith MM, Mucida D, et al. Intestinal monocytes and macrophages are required for T cell polarization in response to *Citrobacter rodentium*. *J Exp Med* (2013) **210**(10):2025–39. doi:10.1084/jem.20130903

188. Diehl GE, Longman RS, Zhang J-X, Breart B, Galan C, Cuesta A, et al. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. *Nature* (2013) **494**(7435):116–20. doi:10.1038/nature11809
189. Ohnmacht C, Pullner A, King SBS, Drexler I, Meier S, Brocker T, et al. Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. *J Exp Med* (2009) **206**(3):549–59. doi:10.1084/jem.20082394
190. Voehringer D, Liang H-E, Locksley RM. Homeostasis and effector function of lymphopenia-induced "memory-like" T cells in constitutively T cell-depleted mice. *J Immunol* (2008) **180**(7):4742–53. doi:10.4049/jimmunol.180.7.4742
191. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**(11):1118–28. doi:10.1038/ni.2419
192. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**(6):1312–26. doi:10.1016/j.cell.2014.11.018
193. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**(6):1327–40. doi:10.1016/j.cell.2014.11.023

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The debate about dendritic cells and macrophages in the kidney

Catherine Gottschalk and Christian Kurts *

Institute of Experimental Immunology, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

The mononuclear phagocyte system includes macrophages and dendritic cells (DCs), which are usually classified by morphology, phenotypical characteristics, and function. In the last decades, large research communities have gathered substantial knowledge on the roles of these cells in immune homeostasis and anti-infectious defense. However, these communities developed to a degree independent from each other, so that the nomenclature and functions of the numerous DC and macrophage subsets overlap, resulting in the present intense debate about the correct nomenclature. This controversy has also reached the field of experimental nephrology. At present, no mutually accepted way to distinguish renal DC and macrophages is available, so that many important roles in acute and chronic kidney disease have been ascribed to both DCs and macrophages. In this perspective article, we discuss the causes and consequences of the overlapping DC–macrophage classification systems, functional roles of DCs and macrophages, and the transferability of recent findings from other disciplines to the renal mononuclear phagocyte system from the nephrologist's point of view.

Keywords: dendritic cells, macrophages, kidney, flow-cytometry, glomerulonephritis

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***Correspondence:**
Christian Kurts, Institute of Experimental Immunology, Rheinische Friedrich-Wilhelms-Universität Bonn, Sigmund-Freud-Str. 25, Bonn D-53127, Germany
ckurts@uni-bonn.de

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Introduction

The current intense debate regarding the classification and nomenclature of dendritic cells (DCs) and macrophages has reached also the field of experimental nephrology. Numerous kidney diseases are immune mediated, such as the different forms of glomerulonephritis, and research over the last years has described important, yet overlapping roles of both cells types.

Macrophages and DCs are often considered distinct cell types based on their morphology and function. Macrophages were defined as large vacuolar cells that are highly phagocytic and modulate immune responses by production of immune mediators (1, 2), whereas DCs were characterized as stellate migratory cells that act as sentinels in non-lymphoid tissues and enter lymphoid tissues upon antigen encounter, present antigen and subsequently activate naïve T lymphocytes (3–5). Following these original descriptions, two research areas developed that more or less independently studied macrophages and DCs. This artificial separation has contributed to the emergence of different names for similar or the same cell types, thereby adding to the current confusion about their identity and function. In particular, advances in multi-color flow cytometry and gene-analyses enabled researchers to define many DC and macrophage subsets by the expression of a variety of surface molecules (6). As cell surface markers are easy to determine, they are widely used to classify mononuclear phagocytes, although they are rather unspecific and their expression patterns in the murine and human systems differ substantially.

Also in the kidney, surface markers and functional parameters have been used to propose several classification systems of mononuclear phagocytes. However, these systems overlap, comparable to the situation in other non-lymphoid organs, resulting in great uncertainty among experimental

nephrologists regarding the correct terminology. Here, we discuss the present state of our knowledge on renal mononuclear phagocytes in health and disease and problems resulting from the current nomenclature debate from a nephrologist point of view.

The Network of Renal DCs and Macrophages

The kidney parenchyma consists of the outer renal cortex and the inner renal medulla. Numerous individual functional units, the nephrons, span both compartments. The cortex contains glomeruli and proximal tubuli of the nephrons, which generate the primary urine. The medulla contains the loop of Henle, which generates a high osmolarity that is required for water reabsorption from the primary urine. The distal tubules end in collecting ducts through which the concentrated urine is transported into the renal pelvis and on through the ureters into the bladder. The space between the tubules is known as tubulointerstitium and contains blood vessels, fibroblasts, and numerous cells of the hematopoietic system that had been classified by pathologists as constituents of the reticuloendothelial system [reviewed in Ref. (7)].

Early immunological studies had classified the tubulointerstitial mononuclear cells as macrophages due to their F4/80 expression (8). During the early 1990s, several groups reported that these tubulointerstitial cells morphologically resembled DCs in humans and rodents (9–12), whereas cells with the typical morphology of macrophages were described to reside mainly in the kidney capsule, the intravasal lumina, and the pelvic wall of healthy kidneys (13). The use of CX_3CR1-reporter mice and live cell imaging illustrated the intricate tubulointerstitial network of dendritiform processes that these cells use to constantly probe the environment, suggestive of DCs in action (14–16). The nomenclature debate intensified when it became clear that the vast majority of renal mononuclear phagocytes possess the phenotype $CD11c^+$ $CD11b^+$ $F4/80^+$ CX_3CR1^+ (17), which allows classification of both macrophages and DCs.

Notably, CX_3CR1 exhibits relative organ specificity for renal mononuclear phagocytes: these cells were >50% reduced in the kidneys, but not in other organs (except the intestine) of CX_3CR1-deficient mice. This may be explained by the comparatively high renal expression of its ligand CX_3CL1 (18). Notably, those CX_3CR1^+ phagocytes that co-express CD11c and exert DC functionality were reduced even by more than 75% (18–20). This may result from an effect of CX_3CR1 on CD11c expression, but this has yet to be shown. Interestingly, CX_3CR1 regulated the numbers of the $CD11c^+$ and the $CD11c^-$ renal mononuclear phagocytes by different mechanisms: it promoted homeostatic and inflammatory recruitment of the former, whereas it prevented *in situ* proliferation of the latter under inflammatory conditions (20). Assuming that CD11c distinguishes renal DCs and macrophages, this difference would be consistent with recent reports that the number of tissue macrophages is regulated by local proliferation (21), whereas DC numbers are usually thought to be regulated by immigration and emigration (22).

The kidney also contains a minor subset of $CD103^+$ DCs, which constitute <5% of all renal $CD11c^+$ phagocytes and lack expression of CX_3CR1, CD11b, and F4/80 (23), whose function currently is unclear. There are neither $CD11b^+$ $CD103^+$ DCs nor plasmacytoid DCs in the healthy kidney (24).

Functionality and Phenotype of Renal Mononuclear Phagocytes

Researchers from both, the DC and the macrophage fields, have investigated kidney mononuclear phagocytes defined by cell surface markers in homeostasis and models of renal disease. Many important roles were shown in models of acute renal injury and in chronic immune-mediated kidney disease (**Table 1**), such as cytokine production or T cell-crosstalk in response to tissue injury or infection (17, 25–33). However, none of these functions is generally accepted to be exclusive for DCs or macrophages. Moreover, many nephrologists trained by the DC and macrophage communities still use CD11c and F4/80 to identify DCs and macrophages, respectively (see **Table 1**), even though 70–90% of renal mononuclear phagocytes co-express these two markers (17), implying that they studied cellular subsets that largely overlap. Also, the tools used for loss-of-function studies cannot clearly discriminate between DCs and macrophages: CD11c–DTR mice are used to deplete kidney DCs, CD11b–DTR mice for depleting kidney macrophages but the expression of CD11c and CD11b on kidney mononuclear phagocytes is too heterogeneous for this black-and-white thinking (34). Clodronate liposomes are used for both purposes (35–38). All kidney mononuclear phagocytes are phagocytic (34) which might render them sensitive to clodronate liposomes.

The consequence of this overlap is well illustrated by two recent studies examining how CX_3CR1 affects renal disease: both studies agreed that mononuclear phagocytes are substantially reduced in the kidneys of CX_3CR1-deficient mice. However, one of them noted a higher susceptibility to renal candidiasis and attributed this to the loss of renal macrophages (19), while the other documented protection against glomerulonephritis and assigned this to the loss of renal DCs (18). A possible explanation for this different classifications is that glomerulonephritis is driven mostly by phagocytes in the kidney cortex, in which glomeruli are located, whereas anti-infectious activity seem to be primarily due to phagocytes in the medulla, through which pathogens enter the kidney (18). Medullary phagocytes express significantly less CD11c than those in the cortex, which may bias their classification as DCs. The causes for these phenotypical and functional differences between medullary and cortical mononuclear phagocytes are unknown, but may result from differences in osmolarity, pH, and oxygen tension between these compartments, to which the mononuclear phagocytes may adapt. This would be in line with the current view that the tissue microenvironment dictates the organ-specific plasticity of macrophages (39, 40), and thus, perhaps also of renal mononuclear phagocytes.

Re-Defining Kidney Mononuclear Phagocyte Nomenclature

The current definitions of renal DCs and macrophages are not mutually exclusive, so that renal mononuclear phagocytes may fulfill the definitions of both cell types simultaneously. This creates

TABLE 1 | Summary of the functions of mononuclear phagocyte subsets in renal diseases, which have been attributed to either renal DC or macrophages, based on marker expression and/or disease attenuation or aggravation after cell depletion.

Disease	Function and associated cell type	Classification of associated cell types
Acute renal injury	**Pro-inflammatory**	**Pro-inflammatory**
Ischemia/re-perfusion	I. TNFa secretion	*DC*
	DC (26, 63, 64)	$CD45^+$, $CD11c^+$, $MHCII^+$, $CD11b^+$, $CD16^+$, $F4/80^+$, $CD68^+$, $CD4^-$, $CD8^-$, $CD205^-$, $33D1^-$, $CD169^-$ $CD204^-$ (26)
	Macrophages (63, 64)	*Macrophages*
	II. Th activation	Sensitive to liposomal dichloromethylene bisphosphonate (clodronate liposome) treatment, $F4/80^+$ (63)
	Macrophages (63)	Sensitive to clodronate liposome treatment (64)
	Anti-inflammatory	**Anti-inflammatory**
	I. Tissue regeneration	*DC*
	DC (33)	Sensitive to clodronate liposome treatment, $CD45^+$, $MHCII^+$,$CD11c^+$, $F4/80^+$ (33)
	Macrophages (67)	Sensitive to clodronate liposome treatment, $CD45^+$, $MHCII^+$, $CD11c^+$ (65)
	II. Suppression of TNFa, IL-6, CXCL2, CCL2 production by IRF4 upregulation	Sensitive to clodronate liposome treatment, $CD11b^+$ (66)
	DC (65)	*Macrophages*
	III. Prevention of renal failure	Sensitive to clodronate liposome treatment, $F4/80^+$ (67)
	DC (66)	
Unilateral ureter obstruction (UUO)	**Pro-inflammatory**	**Pro-inflammatory**
	I. Antigen presentation to $CD4^+$ T cells	*DC*
	DC (27)	$CD11c^+$, T cell stimulatory, phagocytotic (27)
	II. Accumulation of Th17 cells	$CD45^+$, $CD11c^+$, $F4/80^+$, $Ly6C^-$ or $CD45^+$, $CD11c^+$, $F4/80^-$, $Ly6C^-$, sensitive to clodronate liposome treatment (28)
	DC (28)	$CD45^+$ $CD11c^+$, $F4/80^+$ (sensitive to clodronate liposomes) or $F4/80^-$ (not sensitive to clodronate liposomes) (68)
	III. TNFa, TGFb production	*Macrophages*
	DC (28, 68)	$CD45^+$ $F4/80^+$, $CD11c^-$, sensitive to clodronate liposomes (68)
	Macrophages (68)	$CD45^+$, $CD11b^+$, Csfr1R-GFP^+, $CD11c^-$; depletion in CD11b–DTR mice (69)
	IV. Tubular apoptosis	
	DC (68)	
	Macrophages (68)	
	V. Renal fibrosis	
	DC (68, 69)	
	Macrophages (68, 69)	
Adriamycin nephropathy, cisplatin nephropathy, crystal nephropathy	**Pro-inflammatory**	**Pro-inflammatory**
	I. Aggravation of kidney injury in adriamycin-induced nephropathy	*DC*
	Macrophages (25)	*In vitro* studies with bone marrow derived DC; renal $CD45^+$, $CD11c^+$ cells; sensitive to clodronate liposome depletion and diphtheria toxin in CD11c–DTRg mice (29)
	II. IL-1b secretion after inflammasome activation	*Macrophages*
	DC (29)	$CD45^+$, $MHCII^+$, $CD11c^+$, $F4/80^+$, $CD68^+$, $CD204^+$, $CD206^+$, $CD103^-$; morphology, phagocytic capacity, ontogeny (25)
	Anti-inflammatory	**Anti-inflammatory**
	I. Protective against cisplatin nephropathy, induction of IL-10	*DC*
	DC (70)	$CD45^+$, $MHCII^+$, $CD11c^+$, $CD11b^+$, $F4/80^+$; morphology of GFP^+ cells in CD11c–DTRtg mice (70)
Chronic renal disease	**Accumulating**	**Accumulating**
Glomerulonephritides	I. Population changes during nephrotoxic nephritis	*DC*
	DC (17)	$CD11c^+$, $CD11b^+$, $F4/80^+$; morphology, lysosomal content, phagocytic activity, microbicidal effector functions, expression of T cell costimulatory molecules, T cell activation (17)
	Pro-inflammatory	**Pro-inflammatory**
	I. Crescent formation	*DC*
	Macrophages (71)	$MHCII^+$, $CD11c^+$, $F4/80^-$ (72)
	II. T cell infiltration and activation	$MHCII^+$, $CD11c^+$ $CD11b^+$, $CD8^-$, $B220^-$; depletion in CD11c–DTR mice; antigen presentation and T cell activation function (32)
	DC (32, 72)	Chemokine expression by $CD11b^+$ $CD11c^+$ DC was analyzed in lymphoid organs (73)
	III. Chemokine expression	*Macrophages*
	DC (73)	Sensitive to diphtheria toxin in CD11b-DTR mice, $CD68^+$ (71)

(Continued)

TABLE 1 | Continued

Disease	Function and associated cell type	Classification of associated cell types
	Anti-inflammatory	**Anti-inflammatory**
	I. Induction of IL-10 secretion by CD4 T cells	*DC*
	DC (31)	Morphology; MHCII$^+$, CD11c$^+$, CD11b$^+$, sensitive to diphtheria toxin in CD11c-DTR mice (31)
	II. Recruitment of regulatory CXCR6$^+$ iNKT cells	CD45$^+$, CD11c$^+$, depletion in CD11c-luciDTR mice (74)
	DC (74)	
Infection	**Anti-infectious**	**Anti-infectious**
	I. Bacterial clearance	*DC*
	DC (18, 30)	MHCII$^+$, CD45$^+$, CD11c$^+$, CD11b$^+$, F4/80$^+$, CX$_3$CR1$^+$ CD103$^-$; depletion in CD11c–DTR mice (18, 30)
	II. Candida protection	Enrichment by Flt3L administration, sorted by CD11c purification (75)
	Macrophages (19)	*Macrophages*
	III. Response to infectious stimuli, chemokine secretion, migration	MHCII$^+$, F4/80$^+$, CD11b$^+$, CD11c^{lo}; morphology (19)
	DC (75)	

confusion, especially among those nephrologists that are more interested in disease relevance than in semantics. A recent proposal for a unified nomenclature has been based on cellular ontogeny: it proposes an initial division of mononuclear phagocytes into macrophages, monocytes and monocyte-derived cells and DCs (so-called "level 1 nomenclature") (41). This classification was based on the following facts: (1) most adult macrophages in tissues are successors of an embryonic precursor and maintained through self-renewal (42–46), (2) a common monocyte progenitor (cMoP) exists, which gives rise to monocytes (47), and (3) conventional DC (cDC) and plasmacytoid DC but not monocytes or macrophages arise from a common DC precursor (CDP) (48, 49). Thus, tissue-resident macrophages were classified by their origin from embryonic (yolk sac and fetal monocytes)-derived erythro-myeloid progenitors (46, 50) and DC were classified as cells arising from hematopoietic stem cell-derived precursors, identified by genetic tracing via DNGR1 (CLEC9A) (51), which are distinct from monocyte/macrophage precursors. Finally, monocyte-derived cells differentiate from cMoP that can exert macrophage- or DC-like functions and express markers associated with either (41). This classification does not resolve the question whether monocyte-derived macrophages and monocyte-derived DCs are ontogenically distinct or whether one cell type displays high plasticity in different microenvironments. To include cell function, location, and morphology, the authors suggested to add a "level 2" nomenclature to the level 1 classification (41).

While this nomenclature proposal might bring order into the ever increasing numbers DC and macrophage subsets, one major concern remains: without fate mapping tools, the origin of a phagocyte in a given tissue is usually not apparent, so that surrogate markers need to be used. Several markers for distinguishing phagocytes derived from different precursors are currently being discussed, but as we shall see below, they fail to discriminate renal DCs and macrophages.

One of these markers, CD64, alone or in combination with CCR2 or MerTK, has been reported to identify monocyte-derived macrophages and to be able to discriminate DC from non-DC in the intestine, the muscle and spleen (52–55), and the skin (56). DNGR1, when combined with genetic fate mapping technology, was shown to mark CDP and pre-DC (51), whereas Csf1r can be used for fate mapping of yolk sac derived (myb independent) tissue macrophages (46). In the kidney, most mononuclear phagocytes express CD64, low levels of CD11b and high levels of F4/80, which is not the case in other organs. However, 30% of CD64$^+$ cells co-expressed the DNGR1-fate mapper, indicating that CD64 expression, despite the evidence for specificity in other organs, does not differentiate CDP-derived from monocyte-derived cells in the kidney (51). Similarly, another fate mapping study that used Myb and PU.1 dependency for defining CD11b^{hi} monocytes or macrophages and F4/80^{bright} tissue macrophages derived by adult or embryonic hematopoiesis, respectively, found a dual origin in kidney macrophages as well (45). These findings highlight the difficulties when basing cellular classification solely on ontogeny when ontogeny is based on surrogate markers. Furthermore, transferring ontogeny-based nomenclature to human mononuclear phagocytes in tissue might prove impracticable.

A classification approach based on transcriptome analysis reported that CD11c$^+$ MHC II$^+$ cells in the kidney expressed a set of core DC markers characteristic of DCs in non-lymphoid tissues, that is absent from macrophages, including Zbtb64, Flt3, and CCR7 (57). These "core DC markers" had been defined by analyzing cDCs except the CD11b$^+$ non-lymphoid tissue–DC, because of the great heterogeneity of CD11b$^+$ cells. However, these constitute the vast majority of kidney mononuclear phagocytes.

Another classification approach is based on mononuclear phagocyte functionality. However, observed functions generally represent a snapshot of a cell within a specific context and time frame. Demonstrating that a phagocyte performs a given function under certain conditions at a certain time-point does not imply that this is a general feature of this cell. Furthermore, there is no clear demarcation between exclusive DC and macrophage functions. For example, macrophages phagocytose and degrade material. However, under certain conditions DCs do that too, albeit less efficiently [reviewed in Ref. (58)]. On the other hand, DCs classically activate naïve T cells, but macrophages can do

that too, albeit less efficiently (59, 60). Furthermore, the ability to stimulate T cells is difficult to determine on a single cell basis. A recent study differentiated renal mononuclear phagocytes into five phenotypically and functionally distinct populations (34). In that study, mononuclear phagocyte populations were differentiated by CD11c, CD11b, F4/80, CD103, CD14, CD16, and CD64 expression in juvenile and adult mice of different strains. Functional analyses and fate mapping studies were used for further characterization. In line with the complexity of kidney mononuclear phagocyte subsets observed by others and us (17, 45, 51), the study revealed that all subsets expressed CD68 that is usually used to identify macrophages and that all subsets were phagocytic but showed differences in their antigen presentation capacity. Fate mapping experiments identified one population with a dual origin, two populations that were closely related to monocytes, whereas the remaining two were not. Notably, the largest population not only showed the phenotypical and functional characteristics of reparative macrophages (M2) but also had significant antigen presentation function and most likely emigrated from the kidney under inflammatory conditions. Additionally, this population differed significantly between mouse strains, which might explain immunological differences between those strains. The authors concluded that functions are more related to context than separate lineage and suggested their marker combination as an unbiased approach to identify kidney mononuclear phagocyte populations (34). These findings are consistent with recent concepts that macrophage fine differentiation is shaped by the tissue microenvironment (39, 40).

Concluding Remarks

As a consequence of the separate development of the DC and macrophage research communities, the functional and phenotypic definitions of these cell types overlap substantially. Thus, scientists from both communities often study the same cells, perhaps unaware of, or ignoring progress and concepts in the other field. The false assumption that classifying a mononuclear phagocyte as a macrophage implies that it is not a DC, and vice versa, hampers communication between researchers from both fields. Some studies have focused on arguing about subsets and semantics (61), perhaps hoping to "claim territory" for their own communities. This may result in highly citable or controversial publications, but it does not advance our understanding of mononuclear phagocytes, neither in the kidney nor elsewhere.

An overlapping classification system, such as the existing one, is certainly not desirable. An improvement is needed. It is unrealistic to assume that either the DC or the macrophage community will accept the nomenclature of the other field. Drawing a line that segregates mononuclear phagocytes into DCs or macrophages will unlikely be acceptable to both fields. Furthermore, there are currently no unambiguous discriminatory parameters; for any new parameters introduced, exceptions are reported quickly, such as for CD64 and DNGR1-fate tracking in the kidney. Still, an improved classification system is needed. How can we reach a consensus?

First, the purpose of the revised classification system needs to be defined. Clinicians are interested in cellular entities that are useful for diagnostic or therapeutic purposes and translational immunologists often study the functions of cellular subsets. Basic immunologists may favor ontogeny, which is biologically the cleanest and most logical approach. However, mononuclear phagocytes adapt their gene enhancer landscape according to the tissue of residence independently of the precursor they originated from (39), an ontogeny-based nomenclature may lead to different cell types with similar functionality, or to cells of the same name with different functionality depending on the organ they reside in. Moreover, the origin of a mononuclear phagocyte in a given tissue is not obviously apparent, because unique discriminatory parameters are missing. Thus, ontogeny, although theoretically logical, will be difficult to use for routine research. At the end of the day, a classification system needs to be convenient and feasible, or it will not be used.

The late Ralph Steinman remarked "The DC is a functional state" (personal communication). Indeed, at the age of single cell transcriptomics, it becomes clear that several transcriptional programs may run simultaneously in individual mononuclear phagocytes, and confer a spectrum of functionalities that are more or less consistent with the current concepts of a DC, of a macrophage, or both. Current technical advances will undoubtedly allow distinguishing far more functional states of mononuclear phagocytes. In the field of renal immunology, experts coming from the DC and macrophage communities have jointly suggested avoiding the DC–macrophage controversy altogether by referring to mononuclear phagocytes (preferentially using a "catchier" name for these cells), with different degrees of DC- or macrophage-, or other functionalities (62). It remains to be seen whether basic immunologists and scientists studying mononuclear phagocytes in other organs feel that this is useful or not.

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References

1. Mantovani B, Rabinovitch M, Nussenzweig V. Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG). *J Exp Med* (1972) **135**(4):780–92. doi:10.1084/jem.135.4.780
2. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. *Annu Rev Immunol* (1997) **15**:323–50. doi:10.1146/annurev.immunol.15.1.323
3. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* (1973) **137**(5):1142–62. doi:10.1084/jem.137.5.1142
4. Inaba K, Metlay JP, Crowley MT, Steinman RM. Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific, MHC-restricted T cells in situ. *J Exp Med* (1990) **172**(2):631–40. doi:10.1084/jem.172.2.631
5. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* (1991) **9**:271–96. doi:10.1146/annurev.iy.09.040191.001415

6. Hashimoto D, Miller J, Merad M. Dendritic cell and macrophage heterogeneity in vivo. *Immunity* (2011) **35**(3):323–35. doi:10.1016/j.immuni.2011.09.007
7. Saba TM. Physiology and physiopathology of the reticuloendothelial system. *Arch Intern Med* (1970) **126**(6):1031–52. doi:10.1001/archinte.126.6.1031
8. Hume DA, Gordon S. Mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80. Identification of resident macrophages in renal medullary and cortical interstitium and the juxtaglomerular complex. *J Exp Med* (1983) **157**(5):1704–9. doi:10.1084/jem.157.5.1704
9. Markovic-Lipkovski J, Muller CA, Risler T, Bohle A, Muller GA. Association of glomerular and interstitial mononuclear leukocytes with different forms of glomerulonephritis. *Nephrol Dial Transplant* (1990) **5**(1):10–7. doi:10.1093/ndt/5.1.10
10. Cuzic S, Ritz E, Waldherr R. Dendritic cells in glomerulonephritis. *Virchows Arch B Cell Pathol Incl Mol Pathol* (1992) **62**(6):357–63. doi:10.1007/BF02899704
11. Austyn JM, Hankins DF, Larsen CP, Morris PJ, Rao AS, Roake JA. Isolation and characterization of dendritic cells from mouse heart and kidney. *J Immunol* (1994) **152**(5):2401–10.
12. Kaissling B, Le Hir M. Characterization and distribution of interstitial cell types in the renal cortex of rats. *Kidney Int* (1994) **45**(3):709–20. doi:10.1038/ki.1994.95
13. Kaissling B, Hegyi I, Loffing J, Le Hir M. Morphology of interstitial cells in the healthy kidney. *Anat Embryol (Berl)* (1996) **193**(4):303–18. doi:10.1007/BF00186688
14. Soos TJ, Sims TN, Barisoni L, Lin K, Littman DR, Dustin ML, et al. CX3CR1+ interstitial dendritic cells form a contiguous network throughout the entire kidney. *Kidney Int* (2006) **70**(3):591–6. doi:10.1038/sj.ki.5001567
15. Snelgrove SL, Kausman JY, Lo C, Lo C, Ooi JD, Coates PT, et al. Renal dendritic cells adopt a pro-inflammatory phenotype in obstructive uropathy to activate T cells but do not directly contribute to fibrosis. *Am J Pathol* (2012) **180**(1):91–103. doi:10.1016/j.ajpath.2011.09.039
16. Devi S, Li A, Westhorpe CL, Lo CY, Abeynaike LD, Snelgrove SL, et al. Multiphoton imaging reveals a new leukocyte recruitment paradigm in the glomerulus. *Nat Med* (2013) **19**(1):107–12. doi:10.1038/nm.3024
17. Kruger T, Benke D, Eitner F, Lang A, Wirtz M, Hamilton-Williams EE, et al. Identification and functional characterization of dendritic cells in the healthy murine kidney and in experimental glomerulonephritis. *J Am Soc Nephrol* (2004) **15**(3):613–21. doi:10.1097/01.ASN.0000114553.36258.91
18. Hochheiser K, Heuser C, Krause TA, Teteris S, Ilias A, Weisheit C, et al. Exclusive CX3CR1 dependence of kidney DCs impacts glomerulonephritis progression. *J Clin Invest* (2013) **123**(10):4242–54. doi:10.1172/JCI70143
19. Lionakis MS, Swamydas M, Fischer BG, Plantinga TS, Johnson MD, Jaeger M, et al. CX3CR1-dependent renal macrophage survival promotes *Candida* control and host survival. *J Clin Invest* (2013) **123**(12):5035–51. doi:10.1172/JCI71307
20. Engel DR, Krause TA, Snelgrove SL, Thiebes S, Hickey MJ, Boor P, et al. CX3CR1 reduces kidney fibrosis by inhibiting local proliferation of profibrotic macrophages. *J Immunol* (2015) **194**(4):1628–38. doi:10.4049/jimmunol.1402149
21. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, van Rooijen N, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science* (2011) **332**(6035):1284–8. doi:10.1126/science.1204351
22. Liu K, Nussenzweig MC. Origin and development of dendritic cells. *Immunol Rev* (2010) **234**(1):45–54. doi:10.1111/j.0105-2896.2009.00879.x
23. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* (2009) **206**(13):3115–30. doi:10.1084/jem.20091756
24. Kurts C, Panzer U, Anders HJ, Rees AJ. The immune system and kidney disease: basic concepts and clinical implications. *Nat Rev Immunol* (2013) **13**(10):738–53. doi:10.1038/nri3523
25. Cao Q, Wang Y, Wang XM, Lu J, Lee VW, Ye Q, et al. Renal F4/80+ CD11c+ mononuclear phagocytes display phenotypic and functional characteristics of macrophages in health and in adriamycin nephropathy. *J Am Soc Nephrol* (2015) **26**(2):349–63. doi:10.1681/ASN.2013121336
26. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD. Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. *Kidney Int* (2007) **71**(7):619–28. doi:10.1038/sj.ki.5002132
27. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD. Antigen presentation by dendritic cells in renal lymph nodes is linked to systemic and local injury to the kidney. *Kidney Int* (2005) **68**(3):1096–108. doi:10.1111/j.1523-1755.2005.00502.x
28. Dong X, Bachman LA, Miller MN, Nath KA, Griffin MD. Dendritic cells facilitate accumulation of IL-17 T cells in the kidney following acute renal obstruction. *Kidney Int* (2008) **74**(10):1294–309. doi:10.1038/ki.2008.394
29. Mulay SR, Kulkarni OP, Rupanagudi KV, Migliorini A, Darisipudi MN, Vilaysane A, et al. Calcium oxalate crystals induce renal inflammation by NLRP3-mediated IL-1beta secretion. *J Clin Invest* (2013) **123**(1):236–46. doi:10.1172/JCI63679
30. Tittel AP, Heuser C, Ohliger C, Knolle PA, Engel DR, Kurts C. Kidney dendritic cells induce innate immunity against bacterial pyelonephritis. *J Am Soc Nephrol* (2011) **22**(8):1435–41. doi:10.1681/ASN.2010101072
31. Scholz J, Lukacs-Kornek V, Engel DR, Specht S, Kiss E, Eitner F, et al. Renal dendritic cells stimulate IL-10 production and attenuate nephrotoxic nephritis. *J Am Soc Nephrol* (2008) **19**(3):527–37. doi:10.1681/ASN.2007060684
32. Heymann F, Meyer-Schwesinger C, Hamilton-Williams EE, Hammerich L, Panzer U, Kaden S, et al. Kidney dendritic cell activation is required for progression of renal disease in a mouse model of glomerular injury. *J Clin Invest* (2009) **119**(5):1286–97. doi:10.1172/JCI38399
33. Kim MG, Boo CS, Ko YS, Lee HY, Cho WY, Kim HK, et al. Depletion of kidney CD11c+ F4/80+ cells impairs the recovery process in ischaemia/reperfusion-induced acute kidney injury. *Nephrol Dial Transplant* (2010) **25**(9):2908–21. doi:10.1093/ndt/gfq183
34. Kawakami T, Lichtnekert J, Thompson LJ, Karna P, Bouabe H, Hohl TM, et al. Resident renal mononuclear phagocytes comprise five discrete populations with distinct phenotypes and functions. *J Immunol* (2013) **191**(6):3358–72. doi:10.4049/jimmunol.1300342
35. Griffin MD. Mononuclear phagocyte depletion strategies in models of acute kidney disease: what are they trying to tell us? *Kidney Int* (2012) **82**(8):835–7. doi:10.1038/ki.2012.164
36. Tittel AP, Heuser C, Ohliger C, Llanto C, Yona S, Hammerling GJ, et al. Functionally relevant neutrophilia in CD11c diphtheria toxin receptor transgenic mice. *Nat Methods* (2012) **9**(4):385–90. doi:10.1038/nmeth.1905
37. Weisser SB, van Rooijen N, Sly LM. Depletion and reconstitution of macrophages in mice. *J Vis Exp* (2012) (66):4105. doi:10.3791/4105
38. Jung S, Unutmaz D, Wong P, Sano G, De los Santos K, Sparwasser T, et al. In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. *Immunity* (2002) **17**(2):211–20. doi:10.1016/S1074-7613(02)00365-5
39. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**(6):1312–26. doi:10.1016/j.cell.2014.11.018
40. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**(6):1327–40. doi:10.1016/j.cell.2014.11.023
41. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**(8):571–8. doi:10.1038/nri3712
42. Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci* (2011) **14**(9):1142–9. doi:10.1038/nn.2887
43. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) **330**(6005):841–5. doi:10.1126/science.1194637
44. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, et al. Microglia in the adult brain arise from *Ly-6ChiCCR2+* monocytes only under defined host conditions. *Nat Neurosci* (2007) **10**(12):1544–53. doi:10.1038/nn2015
45. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**(6077):86–90. doi:10.1126/science.1219179
46. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2015) **518**(7540):547–51. doi:10.1038/nature13989

47. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* (2013) **14**(8):821–30. doi:10.1038/ni.2638
48. Naik SH, Sathe P, Park HY, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol* (2007) **8**(11):1217–26. doi:10.1038/ni1522
49. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, Manz MG. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol* (2007) **8**(11):1207–16. doi:10.1038/ni1518
50. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol* (2014) **14**(6):392–404. doi:10.1038/nri3671
51. Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* (2013) **154**(4):843–58. doi:10.1016/j.cell.2013.07.014
52. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* (2013) **38**(2):322–35. doi:10.1016/j.immuni.2012.10.016
53. Tamoutounour S, Henri S, Lelouard H, de Bovis B, de Haar C, van der Woude CJ, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol* (2012) **42**(12):3150–66. doi:10.1002/eji.201242847
54. Langlet C, Tamoutounour S, Henri S, Luche H, Ardouin L, Gregoire C, et al. CD64 expression distinguishes monocyte-derived and conventional dendritic cells and reveals their distinct role during intramuscular immunization. *J Immunol* (2012) **188**(4):1751–60. doi:10.4049/jimmunol.1102744
55. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**(11):1118–28. doi:10.1038/ni.2419
56. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* (2013) **39**(5):925–38. doi:10.1016/j.immuni.2013.10.004
57. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* (2012) **13**(9):888–99. doi:10.1038/ni.2370
58. Savina A, Amigorena S. Phagocytosis and antigen presentation in dendritic cells. *Immunol Rev* (2007) **219**:143–56. doi:10.1111/j.1600-065X.2007.00552.x
59. Desmedt M, Rottiers P, Dooms H, Fiers W, Grooten J. Macrophages induce cellular immunity by activating Th1 cell responses and suppressing Th2 cell responses. *J Immunol* (1998) **160**(11):5300–8.
60. Moser M. Regulation of Th1/Th2 development by antigen-presenting cells in vivo. *Immunobiology* (2001) **204**(5):551–7. doi:10.1078/0171-2985-00092
61. Hume DA. Macrophages as APC and the dendritic cell myth. *J Immunol* (2008) **181**(9):5829–35. doi:10.4049/jimmunol.181.9.5829
62. Nelson PJ, Rees AJ, Griffin MD, Hughes J, Kurts C, Duffield J. The renal mononuclear phagocytic system. *J Am Soc Nephrol* (2012) **23**(2):194–203. doi:10.1681/ASN.2011070680
63. Day YJ, Huang L, Ye H, Linden J, Okusa MD. Renal ischemia-reperfusion injury and adenosine 2A receptor-mediated tissue protection: role of macrophages. *Am J Physiol Renal Physiol* (2005) **288**(4):F722–31. doi:10.1152/ajprenal.00378.2004
64. Jo SK, Sung SA, Cho WY, Go KJ, Kim HK. Macrophages contribute to the initiation of ischaemic acute renal failure in rats. *Nephrol Dial Transplant* (2006) **21**(5):1231–9. doi:10.1093/ndt/gfk047
65. Lassen S, Lech M, Rommele C, Mittruecker HW, Mak TW, Anders HJ. Ischemia reperfusion induces IFN regulatory factor 4 in renal dendritic cells, which suppresses postischemic inflammation and prevents acute renal failure. *J Immunol* (2010) **185**(3):1976–83. doi:10.4049/jimmunol.0904207
66. Lech M, Avila-Ferrufino A, Allam R, Segerer S, Khandoga A, Krombach F, et al. Resident dendritic cells prevent postischemic acute renal failure by help of single Ig IL-1 receptor-related protein. *J Immunol* (2009) **183**(6):4109–18. doi:10.4049/jimmunol.0900118
67. Sola A, Weigert A, Jung M, Vinuesa E, Brecht K, Weis N, et al. Sphingosine-1-phosphate signalling induces the production of Lcn-2 by macrophages to promote kidney regeneration. *J Pathol* (2011) **225**(4):597–608. doi:10.1002/path.2982
68. Kitamoto K, Machida Y, Uchida J, Izumi Y, Shiota M, Nakao T, et al. Effects of liposome clodronate on renal leukocyte populations and renal fibrosis in murine obstructive nephropathy. *J Pharmacol Sci* (2009) **111**(3):285–92. doi:10.1254/jphs.09227FP
69. Lin SL, Castano AP, Nowlin BT, Lupher ML Jr, Duffield JS. Bone marrow Ly6Chigh monocytes are selectively recruited to injured kidney and differentiate into functionally distinct populations. *J Immunol* (2009) **183**(10):6733–43. doi:10.4049/jimmunol.0901473
70. Tadagavadi RK, Reeves WB. Renal dendritic cells ameliorate nephrotoxic acute kidney injury. *J Am Soc Nephrol* (2010) **21**(1):53–63. doi:10.1681/ASN.2009040407
71. Duffield JS, Tipping PG, Kipari T, Cailhier JF, Clay S, Lang R, et al. Conditional ablation of macrophages halts progression of crescentic glomerulonephritis. *Am J Pathol* (2005) **167**(5):1207–19. doi:10.1016/S0002-9440(10)61209-6
72. Bagavant H, Deshmukh US, Wang H, Ly T, Fu SM. Role for nephritogenic T cells in lupus glomerulonephritis: progression to renal failure is accompanied by T cell activation and expansion in regional lymph nodes. *J Immunol* (2006) **177**(11):8258–65. doi:10.4049/jimmunol.177.11.8258
73. Ishikawa S, Sato T, Abe M, Nagai S, Onai N, Yoneyama H, et al. Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) by C11b+CD11c+ dendritic cells in murine lupus and preferential chemotaxis of B1 cells towards BLC. *J Exp Med* (2001) **193**(12):1393–402. doi:10.1084/jem.193.12.1393
74. Riedel JH, Paust HJ, Turner JE, Tittel AP, Krebs C, Disteldorf E, et al. Immature renal dendritic cells recruit regulatory CXCR6(+) invariant natural killer T cells to attenuate crescentic GN. *J Am Soc Nephrol* (2012) **23**(12):1987–2000. doi:10.1681/ASN.2012040394
75. Coates PT, Colvin BL, Ranganathan A, Duncan FJ, Lan YY, Shufesky WJ, et al. CCR and CC chemokine expression in relation to Flt3 ligand-induced renal dendritic cell mobilization. *Kidney Int* (2004) **66**(5):1907–17. doi:10.1111/j.1523-1755.2004.00965.x

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frontiers in Immunology

REVIEW
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Guardians of the gut – murine intestinal macrophages and dendritic cells

Mor Gross[1,2], *Tomer-Meir Salame*[1,2] *and Steffen Jung*[1,2]*

[1] Department of Immunology, Weizmann Institute of Science, Rehovot, Israel, [2] Biological Services, Weizmann Institute of Science, Rehovot, Israel

Intestinal mononuclear phagocytes find themselves in a unique environment, most prominently characterized by its constant exposure to commensal microbiota and food antigens. This anatomic setting has resulted in a number of specializations of the intestinal mononuclear phagocyte compartment that collectively contribute the unique steady state immune landscape of the healthy gut, including homeostatic innate lymphoid cells, B, and T cell compartments. As in other organs, macrophages and dendritic cells (DCs) orchestrate in addition the immune defense against pathogens, both in lymph nodes and mucosa-associated lymphoid tissue. Here, we will discuss origins and functions of intestinal DCs and macrophages and their respective subsets, focusing largely on the mouse and cells residing in the lamina propria.

Keywords: gut, dendritic cells, macrophages, homeostasis, inflammation, IBD

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***Correspondence:**
Steffen Jung,
Weizmann Institute of Science,
Department of Immunology, Rehovot
76100, Israel
s.jung@weizmann.ac.il

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The Unique Characteristics of the Gut Landscape

Intestinal mononuclear phagocytes are located in a unique anatomic environment that necessitated the evolution of special functional adaptations of these cells. Exposure to commensal bacteria and harmful pathogens, as well as nutrients and food antigens, in the intestinal lumen force the immune system to continuously weigh tolerogenic and protective immune response. Disruption of this critical and delicate balance can result in devastating inflammatory reactions, e.g., hyper-reactivity to food components (1) or inflammatory bowel diseases (IBD), such as Crohn's disease or ulcerative colitis (2).

Both dendritic cells (DC) and macrophages are found spread throughout the connective tissue that underlies the epithelial layer of the gut, the lamina propria. Moreover, representatives of the two main mononuclear phagocyte families are also located in mucosa-associated lymphoid tissue (MALT), including Peyers' Patches and isolated lymphoid follicles (ILFs) (3). DC and macrophages have distinct, yet complementary roles in maintaining gut homeostasis and immune defense. In keeping with their migratory capacity, DC translocate from the lamina propria via the lymphatics to the gut-draining mesenteric lymph nodes (MsnLNs), where they present antigens to naïve T cells, polarize them toward effector fates, and thus establish the adaptive branch of the immune system (4).

Macrophages, on the other hand, are believed to contribute to the local clearance of bacteria from the tissue, translate alert signals to other immune cells, secrete cytokines to establish the local homeostatic immune cell network, and participate in T cell re-stimulation and maintenance within the lamina propria (5).

DC and macrophages can, as discussed in detail below, be divided into several subpopulations with defined origins, overlapping and distinct surface marker profiles, functions and

TABLE 1 | Mononuclear phagocytes and their respective subsets in the lamina propria of the mouse intestine.

Intestinal mononuclear phagocyte	Main markers (additional markers)	Location	Precursor	Growth/transcription/ environmental factor dependence	Functional specialization	Additional comments	Selected references SI, LI indicate organ of study: small or large intestine
DC	CD103+ CD11b− (CD24+, XCR1+)	Lamina propria, MALT	preDC	Flt-3L Irf8, Id2, Batf-3	Cross-presentation	Equivalent of splenic XCR1+ CD8a+ DC	Edelson et al. (6) SI Ginhoux et al. (7) SI Becker et al. (8) SI Crozat et al. (9) SI Schlitzer et al. (10) SI
	CD103+ CD11b+ (CD24+, Sirpα+)	Lamina propria, MALT	preDC	Flt-3L (partially) Csf-2 (GM-CSF), Irf-4, Notch2, Retinoic acid (ileum)	Required for generation and priming of TH17 cells	More prevalent in ileum	Bogunovic et al. (11) SI, LI Lewis et al. (12) SI, LI Welty et al. (13) SI, LI Schlitzer et al. (10) SI Persson et al. (14) SI, LI Klebanoff et al. (15) SI
	CD103− CD11b+		preDC	Flt-3L, Csf-1 (M-CSF)	Priming of IL-17 and INFγ-producing T cells		Bogunovic et al. (11) SI, LI Cerovic et al. (16) SI Scott et al. (17) SI, LI
	CD103− CD11b-		preDC	Ftl3L	Priming of TH17 *(in vitro)*		Cerovic et al. (16)
Macrophages	CD64+ CX3CR1+ CD11c+ (F4/80+ CD11b+)	Lamina propria	Ly6C+ monocytes	Csf-1 (M-CSF) Csf-2 (GM-CSF) (in colon)			Niess et al. (18) SI Varol et al. (19) SI Bogunovic et al. (11) SI Mortha et al. (20) Cecchini et al. (38)
	CD64+ CX3CR1+ CD11c− (F4/80+ CD11b+)	Lamina propria	Ly6C+ monocytes	Csf-1 (M-CSF) Notch 1/2			Ishifune et al. (21) SI Cecchini et al. (38), SI LI
	CD64+ CX3CR1+ CD169+ (F4/80+ CD11b+)	Crypt proximity	Ly6C+ monocytes	Csf-1 (M-CSF)			Hiemstra et al. (22) LI Cecchini et al. (38), SI LI
	CD64+ CX3CR1+ (F4/80+ CD11b+)	Muscularis layer	Ly6C+ monocytes	Csf-1 (M-CSF)	Communication with neurons		Muller et al. (23) SI, LI Cecchini et al. (38), SI LI

locations. The best characterized DC and macrophage subsets and their key features are summarized in **Table 1**.

With this review, we provide an overview on the characteristics and function of intestinal macrophages and DC in the mouse, including specific roles of their subpopulations. We will discuss distinct origins, roles in maintaining gut homeostasis, and the interactions between these cells and other immune cells. Finally, we will review their communication with their non-immune microenvironment and elaborate on emerging roles of macrophages and DC in inflammation.

Intestinal Macrophages

Macrophages are the most abundant mononuclear phagocytes in the steady-state gut lamina propria (3, 24). Intestinal macrophages are currently best characterized by their expression of CD64, the Fcγ receptor 1 (FcγRI) (25), and the chemokine receptor CX_3CR1 (18), as well as the F4/80 antigen (EGF-like module containing mucin-like hormone receptor-like 1-EMR1) and the integrins CD11b and CD11c (26). Due to the high surface expression levels of the chemokine receptor CX_3CR1 by gut macrophages, these cells can also be readily detected, isolated, and studied *in situ* using intra-vital microscopy on mice harboring a GFP reporter gene inserted into the CX_3CR1 locus (27).

Ontogeny

Like other tissue macrophages (28), also intestinal macrophages are first established before birth from precursors originating in the yolk sac or fetal liver (29). However, unlike macrophages in most other tissues, these embryo-derived cells are replaced in the gut shortly after birth by cells that derive from $Ly6C^+$ blood monocytes (29). The adult monocyte-derived cells display a uniquely short half-life for macrophages (30) indicating their continuous renewal. The monocytic origin of intestinal macrophages was first established in adoptive transfer experiments, involving the transfer of $CX3CR1^{gfp}$ monocyte-precursors and monocytes into CD11c-DTR transgenic mice, whose CD11c-expressing cells, including intestinal macrophages, were depleted by a diphtheria toxin challenge (11, 19, 31). During their differentiation into gut macrophages, monocytes lose Ly6C expression, while other surface markers, such as MHCII, F4/80, CD64, CD11c, and CX3CR1 are up-regulated (25, 32, 33). Moreover, the cells acquire a characteristic anti-inflammatory gene expression profile (32, 34), whose timely establishment and maintenance are critical for gut homeostasis (35). This includes the expression of IL-10, TREM-2, IRAK-M, and tumor necrosis factor (TNF)AIP3 genes, but also of TNFα, which has both pro- and -anti-inflammatory activity (32). Of note, this expression

profile is robust, as it seems to withstand acute challenges, such as the ones associated with oral dextran sulfate sodium (DSS) exposure (32). The molecular cues that drive the "education" of the macrophages in various regions of the gut remain to be defined, but the epithelium is likely to play a role in this process. Epithelial cells could control macrophage differentiation by secretion of immune-regulatory factors, such as thymic stromal lymphopoietin (TSLP), transforming growth factor-β (TGF-β), and prostaglandin E-2 (PGE-2) (36). In addition, recent findings suggested that semaphorin 7A, which is secreted by epithelial cells, contributes to the induction of IL-10 expression by CX_3CR1^+ intestinal macrophages (37). Also, colony-stimulating factor 2 (Csf-1; previously named macrophage colony-stimulating factor, M-CSF) and colony-stimulating factor 2 (Csf-2; previously named granulocyte-macrophage colony-stimulating factor, GM-CSF) play a role in the development of macrophages. Csf-1 is a crucial factor for monocyte development, as Csf-1-deficient osteopetrotic (op/op) mice display reduced levels of $F4/80^+$ cells in the small and large intestine after the first few days of life (28, 38, 39). Csf-2-depleted mice were shown have reduced numbers of $CD11c^+$ colonic macrophages (20).

Of note, $Ly6C^+$ monocytes fail to acquire the characteristic macrophage quiescence during intestinal inflammation, but under this condition respond to local factors that trigger pattern recognition receptors, such as TLRs and NLRs, giving rise to pro-inflammatory macrophages (32). These pro-inflammatory cells, which in acute inflammation outnumber the resident macrophage population, secrete IL-12, IL-23, TNF-α, and inducible nitric oxide synthase (iNOS) (32).

A key suppressor of macrophage-associated inflammation is the IL-10/IL-10 receptor (IL-10R) axis, as mice bearing mutations in IL10-Ra in intestinal $CX3CR1^+$ macrophages developed severe colitis (35) comparable to the pathology reported for IL-10-deficient animals (40). This central critical role of IL-10 in maintaining the non-inflammatory state of macrophages, and thereby, gut homeostasis is also supported by research conducted on samples from humans with loss of function mutations in IL-10R (41). The latter provides an explanation for the severe early onset of colitis observed in pediatric patients harboring non-sense and missense mutations in IL-10R, which reduce IL-10R expression and hamper its signaling cascades (42). Interestingly though, IL-10 production by intestinal macrophages, although also prominent, seems to be redundant for the maintenance of gut homeostasis (35); rather the system seems to rely on alternative IL-10 sources, such as Treg cells (43).

Homeostatic monocyte recruitment to the gut is thought to depend on the chemokine receptor CCR2, as CCR2-deficient mice display less intestinal macrophages and CCR2-deficient intestinal macrophages are underrepresented in mixed bone marrow chimeras (24, 25). The exact factors and mechanisms that ensure homeostatic $Ly6C^+$ monocyte recruitment to the steady state gut are, however, still unknown. While they are likely related to the microbiota exposure of the tissue, analysis of germ-free animals has yielded conflicting results (29, 34, 44, 45). The latter could be due to intestinal embryo-derived macrophages that might persist in the absence of arising competition by an adult monocyte influx.

Macrophage Heterogeneity

Interestingly, emerging evidence suggests that intestinal macrophages are more heterogeneous than previously thought. Monocyte-derived $CD11b^+$ CX_3CR1^+ cells in the gut comprise both $CD11c^+$ and $CD11c^-$ cells. While differential functions of these cells remain to be established, studies into this matter might profit from the recent finding that generation of $CD11c^+$, but not $CD11c^-$ CX_3CR1^+ intestinal macrophages requires Notch signaling (21). A subpopulation of CD169-expressing CX_3CR1^+ macrophages has been reported to be associated with the intestinal crypts (22), although these cells will require further functional characterization. Bogunovic and colleagues recently reported an intriguing $CX3CR1^+$ macrophage subpopulation that resides in the muscularis layer and communicates with enteric neurons to regulate gastrointestinal motility (23). Importantly, we and others have recently shown that macrophages isolated from distinct tissues, such as the liver, lung, brain, and peritoneum, differ considerably with respect to their gene expression profile (46, 47). As expected, this diversity is also prominently reflected in the differential enhancer usage of these cells, as inferred from highly divergent histone modifications (47). Moreover, given that the number of regulatory elements by far exceeds the number of genes (48, 49), this heterogeneity is even more pronounced, including both active and poised enhancer states (47). This applies, albeit to a lesser extent, also to macrophages located in proximal and distal segments of the gut (47). Epigenetic heterogeneity of intestinal macrophages likely reflects monocyte exposure to distinct environmental cues in ileum and colon during their local differentiation (32, 47). In-depth understanding of how these macrophage identities are established, including the hierarchy of induced transcription factors, could yield valuable insights into monocyte differentiation that might be applicable to other tissues and inflammatory settings. PU.1 is a pioneering factor, which induces c-fms transcription and is hence required for macrophage differentiation (50). Intestinal macrophages are furthermore characterized by prominent expression of the Runt-related transcription factor 3 (Runx-3) (47). Interestingly, mice that harbor Runx3 deficiency develop spontaneous colitis (51). Other candidates that might be involved in the establishment of the intestinal macrophage signature are the interferon regulatory factors 4 and 5 (Irf-4, Irf-5), shown to be associated with classical and alternative macrophage activation, respectively (52–54).

Macrophage Interactions with Their Environment

Macrophage Communication with the Epithelial Cell Layer

Pioneering studies by Rescignio and colleagues revealed that certain intestinal mononuclear phagocytes can penetrate the intestinal epithelium by virtue of expression of tight junction proteins and formations of dendritic projections (55). These structures, later termed trans-epithelial dendrites (TEDs) (56), were subsequently ascribed to macrophages expressing CX_3CR1 (18)

and allegedly allow these non-migratory cells to sense, and potentially sample, the luminal content (18, 56). TED formation by macrophages in the terminal region of the ileum was found to be dependent on expression of both CX_3CR1 macrophages and its membrane-tethered ligand CX_3CL1/Fractalkine by selected epithelial cells (57). CX_3CR1-deficient and CX_3CL1-deficient mice were reported to be relatively protected from acute, DSS-induced colitis (58) – a phenotype that might be related to TED formation (57). Likewise, CX_3CR1-deficient mice were shown to display impaired oral tolerance, which was related to impaired IL-10 production by intestinal macrophages, though not their TED formation (59). Finally, there is evidence for a potential role of CX_3CR1^+ macrophages in the capture of luminal bacteria (60) and even the transport of the latter to lymph nodes, at least under conditions of dysbiosis (61). However, the exact definition of macrophage contributions in their native tissue context remains challenging, because it requires their accurate discrimination from closely related and phenotypically similar monocyte-derived DC.

Apart from their role in maintaining intestinal immune homeostasis, gut macrophages also contribute critically to epithelial wound healing. Macrophages associated with the crypts of Lieberkuehn in the colon were reported to assist, following tissue damage, the proliferation and survival of epithelial progenitor cells in a Myd88-dependent manner (62–64). Moreover, in a murine model of acute epithelial regeneration in the colon, activated macrophages supported tissue repair by up-regulating expression of IL-3 and IL-4, while inhibiting secretion of TNF and interferon-γ (IFN-γ) in the lamina propria (3, 65). Macrophages also appear to be able to influence the permeability of the epithelium barrier via the secretion of IL-6 and NO, thereby potentially increasing the invasion of pathogens (66).

Communication with Immune Cells

Macrophages are inferior to DC in their ability to prime naïve T cells (67). This might be due to their rapid degradation of ingested proteins, which impairs their ability to retain antigens for presentation (68). Moreover, at least in steady state, intestinal CX_3CR1^+ macrophages lack expression of CCR7, i.e., the chemokine receptor required for migration to the MsnLNs (25, 69). Rather, the cells that reside in the lamina propria have been proposed to maintain the functionality of FoxP-3$^+$ T regulatory cells that migrated back from the MsnLNs into the tissue (59). Thus, while Treg cell generation of CX_3CR1-deficient mice is unimpaired, these animals harbor reduced Treg cell numbers in the lamina propria, a phenotype that is associated with impaired oral tolerance (59). In light of other data (70), the authors of this study linked the reduced FoxP-3$^+$ Treg cell numbers to impaired production of IL-10 by CX_3CR1^+ macrophages (59). However, the latter might have to be revised, since CX_3CR1^{Cre}:$IL10^{fl/fl}$ mice were shown to harbor unimpaired FoxP-3$^+$ Treg cell numbers (35). Also, interactions between CX_3CR1^+ macrophages and Th17 cells, which are rarely found in intestinal lymphoid tissues and, though primed in the MsnLN, might terminally differentiate in the lamina propria, remain incompletely defined. On one hand, it was shown that intestinal $CD70^{hi}$ CX_3CR1^+ macrophages are activated by commensal-derived ATP and drive the *in vitro* differentiation of Th17 cells (71, 72). On the other hand, intestinal macrophages were reported to counteract Th17 generation that is promoted by $CD103^+CD11b^+$ DC (73, 74). Of note, $CD103^+CD11b^+$ DCs and Th17 cells co-localize in the intestinal tract, as the number of both cells drop from the duodenum to the ileum, and they are scarce in the colon. By contrast, CX_3CR1^+ macrophages and FoxP3$^+$ Treg cells are most abundant in the colon (74).

Recent findings revealed an intriguing cross-talk between intestinal macrophages and innate lymphoid cells (ILC). Thus, in response to luminal stimuli and using a signaling pathway involving the TLR adaptor Myd88, macrophages were shown to secrete IL-1β and in turn induce production of csf-2 by RORγt$^+$ type 3 ILC (20). Mice lacking Csf-2 display reduced numbers of colonic macrophages and DC, associated with a hampered Treg cell compartment (20). Moreover, in a *Citrobacter* infection model CX_3CR1^+ macrophages were shown to promote ILC production of IL-22 via secretion of IL-23 (75), in line with another report (76). Interestingly, CX_3CR1^+ macrophage-derived IL-23 not only induces IL-22 but also seems to concomitantly suppress IL-12 production by $CD103^+$ $CD11b^-$ DC and thereby prevents otherwise detrimental immunopathology (77). Notably, the latter finding provides first evidence for the existence of a direct cross-talk among intestinal mononuclear phagocytes in tissue context, a topic that clearly deserves further study.

Intestinal Dendritic Cells

Dendritic cells are specialized in communicating with T cells, curbing autoreactivity and activating T cell immunity in response to threats. Specifically, DC provide T cells with antigenic peptides that are presented in MHC context, co-stimulation and instructing cytokines that govern T cell polarization into effector cells (67). In order to maintain homeostasis and avoid inflammatory responses toward innocuous antigens, gut DC employ tolerogenic mechanisms that allow them to dampen adaptive immunity. MsnLN- and lamina propria-resident $CD103^+$ DC secrete, for example, retinoic acid (RA) and transforming growth factor-β (TGF-β), which promote the generation of Foxp3$^+$ Treg cells and contribute to the differentiation of plasma cells, which secrete IgA (78, 79).

Classification and Ontogeny

Intestinal DC in mice are characterized by the surface expression of the integrins CD11c (α_X) and CD103 ($\alpha_E\beta7$) (11, 19, 69). More recently, CD24 and Sirpα have been introduced for the better discrimination of DC from macrophages (8, 10). $CD103^+$ DC in the gut arise from dedicated DC precursors, or preDC, and accordingly, mice deficient for fms-related tyrosine kinase-3 receptor (Flt-3) or its ligand Flt-3L have significantly decreased levels of intestinal DC (7, 19). Other, currently though less well-characterized DC progenitors are $\alpha4\beta7^+$ so-called "pre-μDC," which are generated in the bone marrow and were shown to give rise to classical $CD103^+$ DC and $CCR9^+$ plasmacytoid DC (80).

Classical $CD103^+$ DC are divided into two major subpopulations according to their expression of CD11b (α_M) (81). $CD103^+$ $CD11b^+$ DC and $CD103^+$ $CD11b^-$ DC display distinct abundance in small and large intestine, present different additional

surface markers, and require different growth factors for their development (82, 83).

$CD103^+$ $CD11b^+$ DC are developmentally related to $CD11b^+$ $CD8\alpha^-$ splenic DCs (15) and found in the lamina propria of the small and large intestine. They can migrate in CCR7-dependent manner (84) to the MsnLNs, where they present luminal antigens to T cells. $CD103^+$ $CD11b^+$ DCs likely represent a heterogeneous population, as a fraction of them is Csf-2-dependent (3). Development of $CD103^+$ $CD11b^+$ DC, but not of $CD103^+$ $CD11b^-$ DC, is hampered in Csf-2R-deficient mice (85) and when expression of Notch-2 (12, 76) or IRF-4 (14) is impaired. Moreover, $CD103^+$ $CD11b^+$ DC numbers are also reduced in absence of RA and under conditions of vitamin A deprivation (15).

$CD103^+$ $CD11b^-$ DC are more prevalent in lymphoid organs – the Peyer's Patches, MsnLNs, and ILFs (7, 69). However, they can be found also in animals lacking these structures, and are hence not limited to lymphoid tissues (3). Similar to classical $CD8\alpha^+$ DC in the spleen, $CD103^+$ $CD11b^-$ DC depend on the expression of the transcription factors BatF-3 and Irf-8 (6, 15). Like the former, they also express the chemokine receptor XCR1 that has emerged as a universal marker for this DC subset in mouse and human (8, 9). The connection between $CD103^+$ $CD11b^-$ DC and $CD8\alpha^+$ DC is also supported by the fact that the number of $CD103^+$ $CD11b^-$ DC was shown to increase, alongside with splenic $CD8\alpha^+$ DC, in mice that display constitutive β-catenin activation (86). Moreover, like splenic $CD8\alpha^+$ DC (87), also $CD103^+$ $CD11b^-$ DC are specialized in cross-presentation (88).

The exact definition of intestinal DC is complicated, since monocyte-derived cells can acquire phenotypic and functional DC hallmarks. Studies have described a population of $CD103^-CX3CR1^+CD11b^+$ DC, which resides in the lamina propria (11, 16). These cells are CSFR-1 dependent and appear to be derived from $Ly6C^{high}$ monocytes (11). Recent studies also reported that under inflammatory conditions, these $CD103^-CX3CR1^+CD11b^+$ DC expressed CCR7 and migrated in the intestinal lymph, similar to classical intestinal DC, and induced the differentiation of IL-17 and IFN-γ producing T cells (16, 17).

Antigen Sensing and Uptake

$CD103^+$ DC, present in the lamina propria and associated with the intestinal epithelium lining the villi, provide surveillance of the luminal environment (30). They detect foreign and inflammatory signals, acquire and present antigens and interact with T cells by migrating to secondary lymphoid organs (3). Located deep in the core of the villous lamina propria, $CD103^+CD11b^+$ DC would seemingly have limited access to luminal signals, unless antigens or bacteria cross the epithelium or are imported into the lamina propria by other cells, e.g., macrophages, epithelial M cells, or small intestine goblet cells (36, 89, 90). However, lamina propria-resident $CD103^+$ DC were shown to migrate into the epithelial cell layer and capture bacterial antigens (90).

DC Migration

Mucosal T cell priming, arguably one of the primary roles of gut DC, is believed to be restricted to lymphoid tissues (3). Intestinal DC are hence bound to migrate from the lamina propria to the MsnLNs, or within Peyer's Patches into T cell zones. Indeed, $CD103^+$ DC were detected in the intestinal lymph under homeostatic conditions (69, 84). In addition, after systemic BrdU administration, labeled $CD103^+$ DC were found in the lamina propria before they could be discerned in the MsnLNs (30). LN-resident $CD103^+$ DC are thus derived from the tissue and constantly immigrate (30, 91). Interestingly, steady state migration of intestinal $CD103^+$ DC does not appear to be induced by the microbiota or by TLR signaling (92), but may rather depend on a low, tonic release of inflammatory cytokines, or result from spontaneous DC maturation. Nevertheless, entry of $CD103^+$ DC into the MsnLNs is of course considerably enhanced by pro-inflammatory cytokines or TLR ligands (93, 94). Migration of intestinal DC depends on CCR7, both in steady state and under inflammatory conditions. Accordingly, CCR7 expression is up-regulated in DC before their migration from the tissue into the MsnLN (84) and CCR7 deficient DC fail to migrate (69, 84, 95). Moreover, it was recently shown that DC can also migrate from the lamina propria into the epithelial layer (90) and can thus gain direct access to antigen and luminal bacteria. Hence, following challenge with *Salmonella*, accumulation of the bacteria was first observed in DC of the epithelial fraction and only subsequently in DC in the lamina propria (90).

DC and the Epithelium

DC intimately interact with the epithelial layer of the intestine by a variety of mechanisms. Small intestinal goblet cells were shown to transfer small soluble antigens from the intestinal lumen to $CD103^+$ DC (89). Chemokines secreted by enterocytes in response to TLR ligand exposure can induce the above-mentioned relocation of lamina propria DC to the epithelium (90). In addition, it is becoming more and more evident that epithelial cells play a critical role in maintaining DC in a tolerogenic state, compatible with gut homeostasis. Epithelial and stromal cells secrete factors, which are thought to induce DC tolerance, such as RA, TGF-β, PGE-2, and TSLP (3, 82, 96–99). In parallel to ILC (20), intestinal epithelial cells regulate retinal dehydrogenase (RALDH) expression by $CD103^+$ DC that the cells need to metabolize retinoids. Specifically, epithelial cells express a critical cytosolic retinoid chaperone, the cellular retinol binding protein II, which is required for *in vivo* imprinting of gut DC by lumenal retinoids (99, 100). Supporting this notion, the *in vitro* co-culture of bone marrow- or spleen-derived DC with epithelial cells results in the up-regulation of CD103 and RALDH, together with TGF-β imprinted homing potential on T cells (101–103). These data establish the potential of intestinal epithelial cells to educate intestinal DC, although further *in vivo* studies and higher resolution, with respect to cell subsets, are required to better elucidate the underlying mechanisms.

DC Communication with Intestinal T Cells

Intestinal $CD103^+$ DC, found in lamina propria, Peyer's Patches, and the MsnLNs program T cells to express the gut-homing factors CCR9 and $\alpha4\beta7$ integrin (101, 104, 105). Concomitantly, DC can also induce the development of $FoxP\text{-}3^+$ and IL-10 producing

Treg cells (106) and prime Th17 cells (17, 107, 108). The majority of these DC-governed priming events require TGF-β signaling and RA, which are generated in the DC by enzymatic conversion of all-trans-retinal, a derivative of vitamin A, using RALDH2 (101, 109, 110). Indeed, RA has emerged as the critical conditioning factor for intestinal DC, as vitamin A is crucial for the activity of the enzyme RALDH in DC. Without RALDH, the ability of DC to imprint T cells is hampered, and restored only after vitamin A administration (111). The balance between RA and TGF-β levels seems to determine the fate of Treg cells primed by DC, as presence of both RA and TGF-β favor the development of FoxP-3$^+$ cells, while RA induces the generation of IL-10 producing T cells (106).

Other enzymes that influence the outcome of T cell priming are indoleamine 2,3 dioxygenase (IDO) and TSLP. IDO is expressed also by DC in other tissues and was shown to inhibit the development of effector T cells and promote Treg cell generation (112, 113). TSLP is, as mentioned above, secreted by epithelial cells, but also by the intestinal DC, themselves. In the presence

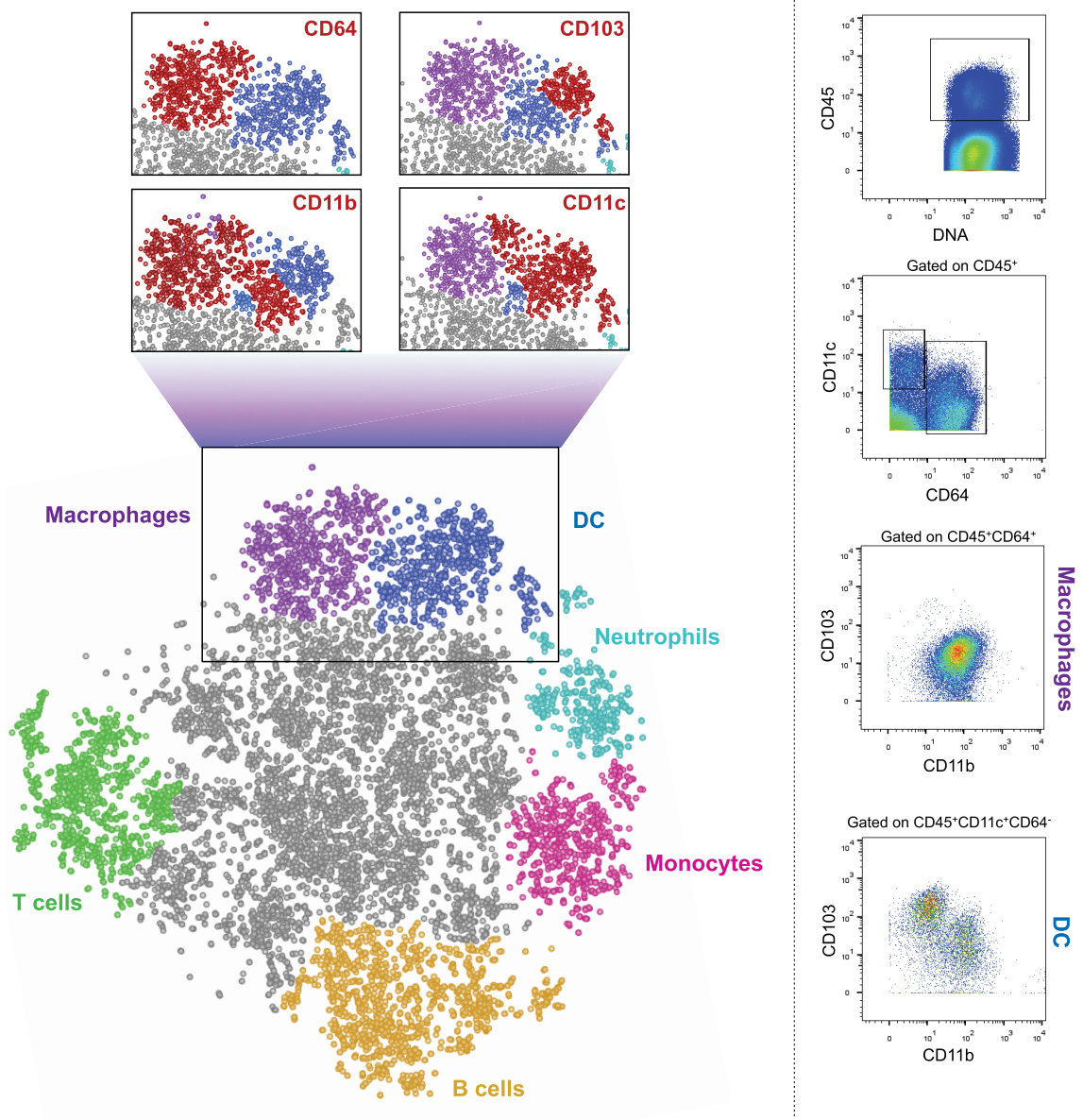

FIGURE 1 | CyTOF analysis of CD45$^+$ cells from murine colon. Cells were isolated from colon of 6–9 weeks old WT female C57Bl/6 mice and stained with a panel of 26 cell surface markers. The results were gated for live, single, CD45$^+$ cells. Bh-SNE analysis and clustering were performed by Accense (http://www.cellaccense.com/) and the results were processed by GIMP. Colors indicate high levels of the following markers: green – TCRβ, CD3e (T cells), Orange – B220 (B cells), light blue – Ly6G (granulocytes), pink –Ly6C (monocytes), purple – CD64, F4/80 (macrophages), blue – clustered by Accence, different DC populations, gray – non-identified or non-specific cells. Red populations in zoom-in black squares indicate high levels of the marker written. Representative of at least four separate, independent experiments.

of TSLP, Th17 responses are restricted due to a reduced ability to produce IL-17, and Treg cell differentiation is up-regulated (107). The ability of the intestinal DC compartment to generate Th17 cells seems to be associated with $CD103^+$ $CD11b^+$ DC, as the frequency of Th17 cells is reduced in mice lacking these DC due to either IRF-4 or Notch-deficiency (10, 12, 14), or as a result of conditional ablation of this DC subset (13). Interestingly though, a recent study showed that also another subpopulation of DC, i.e. $CCR2^+$ $CD103^-$ $CD11b^+$ DC can induce IL-17a production in $CD4^+$ T cells and effectively prime Th17 cells, probably via IL-12/IL-23p40 secretion (17).

Intestinal DC, Inflammation, and Immune Response

In steady state, intestinal DC are probably mainly tolerogenic. Under inflammatory conditions, however, they can become highly effective T cell activators (114). Induction of experimental colitis results in the accumulation of $CD103^+$ DC with an inflammatory profile in the MsnLNs (114). These DC express less RALDH and TGF-β and instead of promoting Treg cell formation, now induce Th1 inflammatory responses (114). While Th17 polarization might be carried out by $CD103^+$ $CD11b^+$ DC (12), differentiation of $CD8^+$ effector T cells under inflammatory conditions seems to be dependent on $CD103^+$ $CD11b^-$ $CD8\alpha^+$ DC that migrated into the lymph (88).

Flagellin stimulation causes TLR-5^+ $CD103^+$ DC in the small intestine to promote differentiation of Th17 cells and secrete IL-23, which in turn induces IL-22 production by ILC3 and subsequent epithelial up-regulation of antibacterial peptides (115).

In summary, DC are major players in maintaining homeostasis in the intestine. While tolerogenic at steady state, under inflammatory conditions they tip the scales and activate the immune system. They can migrate between different compartments of the intestine – from the lamina propria to the epithelium and into the MsnLNs – and execute different immune responses in each tissue. Further research regarding the location of DC, their functions and characteristics should shed new light on the role of these cells in the intestine.

Concluding Remarks and a Glimpse to the Future

In summary, macrophages and DC critically contribute to intestinal homeostasis and immune defense. Both cellular compartments have been subdivided into discrete subpopulations, which though currently mainly phenotypically defined, in some cases have been assigned distinct activities. The challenge ahead is to better define precise roles of these subsets both in health and under inflammatory conditions, first in the mouse but then also in the human. This task is complicated by the fact that many of the used markers used to distinguish between subpopulations of DC and macrophages are shared by the two types of mononuclear phagocytes. Moreover, under inflammatory conditions monocyte-derived cells further blur the picture. Collectively, this highlights the need to define cells by multiple parameters, including both surface and intracellular markers. Single cell transcriptome analysis is likely to help with this task (116, 117). However, classic flow cytometry analysis using fluorescent dye-coupled antibodies allows only a very limited simultaneous panel of markers due to the few dyes available and the spectral overlap of their emission. This problem might, in the near future, be solved by spectral cytometry systems that use ultrafast optical spectroscopy combined with flow cytometry to differentiate between the emission curves of different fluorophores, thus enabling the use of dozens of antibodies in one sample (118). Moreover, a new cell analyzer has been introduced, which uses mass cytometry instead of flow cytometry and is termed cytometry by Time-Of-Flight, or CyTOF (119). Instead of conjugations to fluorophores, this machine uses conjugations to heavy metal isotopes. Such metals do not exist naturally in the cells, so background is insignificant. The stained cells are injected into the CyTOF and are evaporated in a plasma chamber. The metals are ionized, hit the TOF detector, and their mass is measured, allowing the machine to determine the expression levels of the markers on each cell. This multiple-parameter approach enables to explore entire immune cell populations and subpopulations from the same tissue. As exemplified in **Figure 1**, such global analysis methods might well hold the key for the better definition and understanding of the cellular make-up of the intestine. No doubt, that with the recent development in the fields of cell cytometry and RNA sequencing, more pieces of this complex puzzle of the characteristics and roles of mononuclear phagocytes in the gut will be detected and put in place.

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References

1. Meresse B, Malamut G, Cerf-Bensussan N. Celiac disease: an immunological jigsaw. *Immunity* (2012) **36**:907–19. doi:10.1016/j.immuni.2012.06.006
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* (2007) **448**:427–34. doi:10.1038/nature06005
3. Farache J, Zigmond E, Shakhar G, Jung S. Contributions of dendritic cells and macrophages to intestinal homeostasis and immune defense. *Immunol Cell Biol* (2013) **91**:232–9. doi:10.1038/icb.2012.79
4. Bekiaris V, Persson EK, Agace WW. Intestinal dendritic cells in the regulation of mucosal immunity. *Immunol Rev* (2014) **260**:86–101. doi:10.1111/imr.12194
5. Zigmond E, Jung S. Intestinal macrophages: well educated exceptions from the rule. *Trends Immunol* (2013) **34**:162–8. doi:10.1016/j.it.2013.02.001
6. Edelson BT, Kc W, Juang R, Kohyama M, Benoit LA, Klekotka PA, et al. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J Exp Med* (2010) **207**:823–36. doi:10.1084/jem.20091627
7. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* (2009) **206**:3115–30. doi:10.1084/jem.20091756
8. Becker M, Guttler S, Bachem A, Hartung E, Mora A, Jakel A, et al. Ontogenic, phenotypic, and functional characterization of XCR1(+) dendritic cells leads to a consistent classification of intestinal dendritic cells based on the expression of

XCR1 and SIRPalpha. *Front Immunol* (2014) **5**:326. doi:10.3389/fimmu.2014.00326
9. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, Ventre E, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**:1283–92. doi:10.1084/jem.20100223
10. Schlitzer A, Mcgovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* (2013) **38**:970–83. doi:10.1016/j.immuni.2013.04.011
11. Bogunovic M, Ginhoux F, Helft J, Shang L, Hashimoto D, Greter M, et al. Origin of the lamina propria dendritic cell network. *Immunity* (2009) **31**:513–25. doi:10.1016/j.immuni.2009.08.010
12. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* (2011) **35**:780–91. doi:10.1016/j.immuni.2011.08.013
13. Welty NE, Staley C, Ghilardi N, Sadowsky MJ, Igyarto BZ, Kaplan DH. Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. *J Exp Med* (2013) **210**:2011–24. doi:10.1084/jem.20130728
14. Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hagerbrand K, Marsal J, et al. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* (2013) **38**:958–69. doi:10.1016/j.immuni.2013.03.009
15. Klebanoff CA, Spencer SP, Torabi-Parizi P, Grainger JR, Roychoudhuri R, Ji Y, et al. Retinoic acid controls the homeostasis of pre-cDC-derived splenic and intestinal dendritic cells. *J Exp Med* (2013) **210**:1961–76. doi:10.1084/jem.20122508
16. Cerovic V, Houston SA, Scott CL, Aumeunier A, Yrlid U, Mowat AM, et al. Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells. *Mucosal Immunol* (2013) **6**:104–13. doi:10.1038/mi.2012.53
17. Scott CL, Bain CC, Wright PB, Sichien D, Kotarsky K, Persson EK, et al. CCR2CD103 intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunol* (2014) **8**(2):327–39. doi:10.1038/mi.2014.70
18. Niess JH, Brand S, Gu X, Landsman L, Jung S, Mccormick BA, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* (2005) **307**:254–8. doi:10.1126/science.1102901
19. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity* (2009) **31**:502–12. doi:10.1016/j.immuni.2009.06.025
20. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science* (2014) **343**:1249288. doi:10.1126/science.1249288
21. Ishifune C, Maruyama S, Sasaki Y, Yagita H, Hozumi K, Tomita T, et al. Differentiation of CD11c+ CX3CR1+ cells in the small intestine requires notch signaling. *Proc Natl Acad Sci U S A* (2014) **111**:5986–91. doi:10.1073/pnas.1401671111
22. Hiemstra IH, Beijer MR, Veninga H, Vrijland K, Borg EG, Olivier BJ, et al. The identification and developmental requirements of colonic CD169(+) macrophages. *Immunology* (2014) **142**:269–78. doi:10.1111/imm.12251
23. Muller PA, Koscso B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* (2014) **158**:300–13. doi:10.1016/j.cell.2014.04.050
24. Bain CC, Mowat AM. Intestinal macrophages – specialised adaptation to a unique environment. *Eur J Immunol* (2011) **41**:2494–8. doi:10.1002/eji.201141714
25. Tamoutounour S, Henri S, Lelouard H, De Bovis B, De Haar C, Van Der Woude CJ, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol* (2012) **42**:3150–66. doi:10.1002/eji.201242847
26. Pabst O, Bernhardt G. The puzzle of intestinal lamina propria dendritic cells and macrophages. *Eur J Immunol* (2010) **40**:2107–11. doi:10.1002/eji.201040557
27. Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, Sher A, et al. Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* (2000) **20**:4106–14. doi:10.1128/MCB.20.11.4106-4114.2000
28. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol* (2014) **14**:392–404. doi:10.1038/nri3671
29. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) **15**:929–37. doi:10.1038/ni.2967
30. Jaensson E, Uronen-Hansson H, Pabst O, Eksteen B, Tian J, Coombes JL, et al. Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. *J Exp Med* (2008) **205**:2139–49. doi:10.1084/jem.20080414
31. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* (2007) **204**:171–80. doi:10.1084/jem.20061011
32. Zigmond E, Varol C, Farache J, Elmaliah E, Satpathy AT, Friedlander G, et al. Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. *Immunity* (2012) **37**:1076–90. doi:10.1016/j.immuni.2012.08.026
33. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. *Mucosal Immunol* (2013) **6**:498–510. doi:10.1038/mi.2012.89
34. Rivollier A, He J, Kole A, Valatas V, Kelsall BL. Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *J Exp Med* (2012) **209**:139–55. doi:10.1084/jem.20101387
35. Zigmond E, Bernshtein B, Friedlander G, Walker CR, Yona S, Kim KW, et al. Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. *Immunity* (2014) **40**:720–33. doi:10.1016/j.immuni.2014.03.012
36. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol* (2008) **8**:411–20. doi:10.1038/nri2316
37. Kang S, Okuno T, Takegahara N, Takamatsu H, Nojima S, Kimura T, et al. Intestinal epithelial cell-derived semaphorin 7A negatively regulates development of colitis via alphavbeta1 integrin. *J Immunol* (2012) **188**:1108–16. doi:10.4049/jimmunol.1102084
38. Cecchini MG, Dominguez MG, Mocci S, Wetterwald A, Felix R, Fleisch H, et al. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Development* (1994) **120**:1357–72.
39. Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood* (2002) **99**:111–20. doi:10.1182/blood.V99.1.111
40. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* (1993) **75**:263–74. doi:10.1016/0092-8674(93)80068-P
41. Shouval DS, Biswas A, Goettel JA, Mccann K, Conaway E, Redhu NS, et al. Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. *Immunity* (2014) **40**:706–19. doi:10.1016/j.immuni.2014.03.011
42. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N Engl J Med* (2009) **361**:2033–45. doi:10.1056/NEJMoa0907206
43. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* (2008) **28**:546–58. doi:10.1016/j.immuni.2008.02.017
44. Niess JH, Adler G. Enteric flora expands gut lamina propria CX3CR1+ dendritic cells supporting inflammatory immune responses under normal and inflammatory conditions. *J Immunol* (2010) **184**:2026–37. doi:10.4049/jimmunol.0901936
45. Ueda Y, Kayama H, Jeon SG, Kusu T, Isaka Y, Rakugi H, et al. Commensal microbiota induce LPS hyporesponsiveness in colonic macrophages via the production of IL-10. *Int Immunol* (2010) **22**:953–62. doi:10.1093/intimm/dxq449
46. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the

identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**:1118–28. doi:10.1038/ni.2419
47. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
48. Ghisletti S, Barozzi I, Mietton F, Polletti S, De Santa F, Venturini E, et al. Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. *Immunity* (2010) **32**:317–28. doi:10.1016/j.immuni.2010.02.008
49. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* (2010) **38**:576–89. doi:10.1016/j.molcel.2010.05.004
50. Sasmono RT, Oceandy D, Pollard JW, Tong W, Pavli P, Wainwright BJ, et al. A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. *Blood* (2003) **101**:1155–63. doi:10.1182/blood-2002-02-0569
51. Brenner O, Levanon D, Negreanu V, Golubkov O, Fainaru O, Woolf E, et al. Loss of Runx3 function in leukocytes is associated with spontaneously developed colitis and gastric mucosal hyperplasia. *Proc Natl Acad Sci U S A* (2004) **101**:16016–21. doi:10.1073/pnas.0407180101
52. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* (2010) **11**:936–44. doi:10.1038/ni.1920
53. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* (2011) **12**:231–8. doi:10.1038/ni.1990
54. Okabe Y, Medzhitov R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell* (2014) **157**:832–44. doi:10.1016/j.cell.2014.04.016
55. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* (2001) **2**:361–7. doi:10.1038/86373
56. Vallon-Eberhard A, Landsman L, Yogev N, Verrier B, Jung S. Transepithelial pathogen uptake into the small intestinal lamina propria. *J Immunol* (2006) **176**:2465–9. doi:10.4049/jimmunol.176.4.2465
57. Kim KW, Vallon-Eberhard A, Zigmond E, Farache J, Shezen E, Shakhar G, et al. In vivo structure/function and expression analysis of the CX3C chemokine fractalkine. *Blood* (2011) **118**:e156–67. doi:10.1182/blood-2011-04-348946
58. Kostadinova FI, Baba T, Ishida Y, Kondo T, Popivanova BK, Mukaida N. Crucial involvement of the CX3CR1-CX3CL1 axis in dextran sulfate sodium-mediated acute colitis in mice. *J Leukoc Biol* (2010) **88**:133–43. doi:10.1189/jlb.1109768
59. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity* (2011) **34**:237–46. doi:10.1016/j.immuni.2011.01.016
60. Hapfelmeier S, Muller AJ, Stecher B, Kaiser P, Barthel M, Endt K, et al. Microbe sampling by mucosal dendritic cells is a discrete, MyD88-independent step in DeltainvG S. *Typhimurium colitis*. *J Exp Med* (2008) **205**:437–50. doi:10.1084/jem.20070633
61. Diehl GE, Longman RS, Zhang JX, Breart B, Galan C, Cuesta A, et al. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. *Nature* (2013) **494**:116–20. doi:10.1038/nature11809
62. Slavin J, Nash JR, Kingsnorth AN. Effect of transforming growth factor beta and basic fibroblast growth factor on steroid-impaired healing intestinal wounds. *Br J Surg* (1992) **79**:69–72. doi:10.1002/bjs.1800790124
63. Fukata M, Michelsen KS, Eri R, Thomas LS, Hu B, Lukasek K, et al. Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *Am J Physiol Gastrointest Liver Physiol* (2005) **288**:G1055–65. doi:10.1152/ajpgi.00328.2004
64. Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc Natl Acad Sci U S A* (2005) **102**:99–104. doi:10.1073/pnas.0405979102
65. Seno H, Miyoshi H, Brown SL, Geske MJ, Colonna M, Stappenbeck TS. Efficient colonic mucosal wound repair requires Trem2 signaling. *Proc Natl Acad Sci U S A* (2009) **106**:256–61. doi:10.1073/pnas.0803343106
66. Du Plessis J, Vanheel H, Janssen CE, Roos L, Slavik T, Stivaktas PI, et al. Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* (2013) **58**:1125–32. doi:10.1016/j.jhep.2013.01.038
67. Mildner A, Jung S. Development and function of dendritic cell subsets. *Immunity* (2014) **40**:642–56. doi:10.1016/j.immuni.2014.04.016
68. Delamarre L, Pack M, Chang H, Mellman I, Trombetta ES. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science* (2005) **307**:1630–4. doi:10.1126/science.1108003
69. Schulz O, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW, et al. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med* (2009) **206**:3101–14. doi:10.1084/jem.20091925
70. Murai M, Turovskaya O, Kim G, Madan R, Karp CL, Cheroutre H, et al. Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. *Nat Immunol* (2009) **10**:1178–84. doi:10.1038/ni.1791
71. Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, et al. ATP drives lamina propria T(H)17 cell differentiation. *Nature* (2008) **455**:808–12. doi:10.1038/nature07240
72. Medina-Contreras O, Geem D, Laur O, Williams IR, Lira SA, Nusrat A, et al. CX3CR1 regulates intestinal macrophage homeostasis, bacterial translocation, and colitogenic Th17 responses in mice. *J Clin Invest* (2011) **121**:4787–95. doi:10.1172/JCI59150
73. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* (2007) **8**:1086–94. doi:10.1038/ni1511
74. Denning TL, Norris BA, Medina-Contreras O, Manicassamy S, Geem D, Madan R, et al. Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. *J Immunol* (2011) **187**:733–47. doi:10.4049/jimmunol.1002701
75. Longman RS, Diehl GE, Victorio DA, Huh JR, Galan C, Miraldi ER, et al. CX(3)CR1(+) mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. *J Exp Med* (2014) **211**:1571–83. doi:10.1084/jem.20140678
76. Satpathy AT, Briseno CG, Lee JS, Ng D, Manieri NA, Kc W, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol* (2013) **14**:937–48. doi:10.1038/ni.2679
77. Aychek T, Mildner A, Yona S, Kim KW, Lampl N, Reich-Zeliger S, et al. IL-23-mediated mononuclear phagocyte crosstalk protects mice from *Citrobacter rodentium*-induced colon immunopathology. *Nat Commun* (2015) **6**:6525. doi:10.1038/ncomms7525
78. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* (2007) **204**:1775–85. doi:10.1084/jem.20070602
79. Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* (2008) **8**:435–46. doi:10.1038/nri2335
80. Zeng R, Oderup C, Yuan R, Lee M, Habtezion A, Hadeiba H, et al. Retinoic acid regulates the development of a gut-homing precursor for intestinal dendritic cells. *Mucosal Immunol* (2012) **6**:847–56. doi:10.1038/mi.2012.123
81. Milling S, Yrlid U, Cerovic V, Macpherson G. Subsets of migrating intestinal dendritic cells. *Immunol Rev* (2010) **234**:259–67. doi:10.1111/j.0105-2896.2009.00866.x
82. Pulendran B, Tang H, Denning TL. Division of labor, plasticity, and crosstalk between dendritic cell subsets. *Curr Opin Immunol* (2008) **20**:61–7. doi:10.1016/j.coi.2007.10.009
83. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. *Nat Rev Immunol* (2014) **14**:667–85. doi:10.1038/nri3738
84. Jang MH, Sougawa N, Tanaka T, Hirata T, Hiroi T, Tohya K, et al. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. *J Immunol* (2006) **176**:803–10. doi:10.4049/jimmunol.176.2.803

85. Cerovic V, Bain CC, Mowat AM, Milling SW. Intestinal macrophages and dendritic cells: what's the difference? *Trends Immunol* (2014) **35**:270–7. doi:10.1016/j.it.2014.04.003
86. Cohen SB, Smith NL, Mcdougal C, Pepper M, Shah S, Yap GS, et al. Beta-catenin signaling drives differentiation and proinflammatory function of IRF8-dependent dendritic cells. *J Immunol* (2014) **194**(1):210–22. doi:10.4049/jimmunol.1402453
87. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* (2000) **192**:1685–96. doi:10.1084/jem.192.12.1685
88. Cerovic V, Houston SA, Westlund J, Utriainen L, Davison ES, Scott CL, et al. Lymph-borne CD8alpha dendritic cells are uniquely able to cross-prime CD8 T cells with antigen acquired from intestinal epithelial cells. *Mucosal Immunol* (2014) **8**(1):38–48. doi:10.1038/mi.2014.40
89. McDole JR, Wheeler LW, Mcdonald KG, Wang B, Konjufca V, Knoop KA, et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* (2012) **483**:345–9. doi:10.1038/nature10863
90. Farache J, Koren I, Milo I, Gurevich I, Kim KW, Zigmond E, et al. Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity* (2013) **38**:581–95. doi:10.1016/j.immuni.2013.01.009
91. Bimczok D, Sowa EN, Faber-Zuschratter H, Pabst R, Rothkotter HJ. Site-specific expression of CD11b and SIRPalpha (CD172a) on dendritic cells: implications for their migration patterns in the gut immune system. *Eur J Immunol* (2005) **35**:1418–27. doi:10.1002/eji.200425726
92. Wilson NS, Young LJ, Kupresanin F, Naik SH, Vremec D, Heath WR, et al. Normal proportion and expression of maturation markers in migratory dendritic cells in the absence of germs or toll-like receptor signaling. *Immunol Cell Biol* (2008) **86**:200–5. doi:10.1038/sj.icb.7100125
93. Sierro F, Dubois B, Coste A, Kaiserlian D, Kraehenbuhl JP, Sirard JC. Flagellin stimulation of intestinal epithelial cells triggers CCL20-mediated migration of dendritic cells. *Proc Natl Acad Sci U S A* (2001) **98**:13722–7. doi:10.1073/pnas.241308598
94. Yrlid U, Milling SW, Miller JL, Cartland S, Jenkins CD, Macpherson GG. Regulation of intestinal dendritic cell migration and activation by plasmacytoid dendritic cells, TNF-alpha and type 1 IFNs after feeding a TLR7/8 ligand. *J Immunol* (2006) **176**:5205–12. doi:10.4049/jimmunol.176.9.5205
95. Forster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol* (2008) **8**:362–71. doi:10.1038/nri2297
96. Rimoldi M, Chieppa M, Salucci V, Avogadri F, Sonzogni A, Sampietro GM, et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol* (2005) **6**:507–14. doi:10.1038/ni1192
97. Zeuthen LH, Fink LN, Frokiaer H. Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta. *Immunology* (2008) **123**:197–208. doi:10.1111/j.1365-2567.2007.02687.x
98. Iliev ID, Spadoni I, Mileti E, Matteoli G, Sonzogni A, Sampietro GM, et al. Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. *Gut* (2009) **58**:1481–9. doi:10.1136/gut.2008.175166
99. Stock A, Booth S, Cerundolo V. Prostaglandin E2 suppresses the differentiation of retinoic acid-producing dendritic cells in mice and humans. *J Exp Med* (2011) **208**:761–73. doi:10.1084/jem.20101967
100. McDonald KG, Leach MR, Brooke KW, Wang C, Wheeler LW, Hanly EK, et al. Epithelial expression of the cytosolic retinoid chaperone cellular retinol binding protein II is essential for in vivo imprinting of local gut dendritic cells by lumenal retinoids. *Am J Pathol* (2012) **180**:984–97. doi:10.1016/j.ajpath.2011.11.009
101. Johansson-Lindbom B, Svensson M, Pabst O, Palmqvist C, Marquez G, Forster R, et al. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* (2005) **202**:1063–73. doi:10.1084/jem.20051100
102. Edele F, Molenaar R, Gutle D, Dudda JC, Jakob T, Homey B, et al. Cutting edge: instructive role of peripheral tissue cells in the imprinting of T cell homing receptor patterns. *J Immunol* (2008) **181**:3745–9. doi:10.4049/jimmunol.181.6.3745
103. Iliev ID, Mileti E, Matteoli G, Chieppa M, Rescigno M. Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning. *Mucosal Immunol* (2009) **2**:340–50. doi:10.1038/mi.2009.13
104. Mora JR, Bono MR, Manjunath N, Weninger W, Cavanagh LL, Rosemblatt M, et al. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* (2003) **424**:88–93. doi:10.1038/nature01726
105. Annacker O, Coombes JL, Malmstrom V, Uhlig HH, Bourne T, Johansson-Lindbom B, et al. Essential role for CD103 in the T cell-mediated regulation of experimental colitis. *J Exp Med* (2005) **202**:1051–61. doi:10.1084/jem.20040662
106. Bakdash G, Vogelpoel LT, Van Capel TM, Kapsenberg ML, De Jong EC. Retinoic acid primes human dendritic cells to induce gut-homing, IL-10-producing regulatory T cells. *Mucosal Immunol* (2014) **8**(2):265–78. doi:10.1038/mi.2014.64
107. Spadoni I, Iliev ID, Rossi G, Rescigno M. Dendritic cells produce TSLP that limits the differentiation of Th17 cells, fosters Treg development, and protects against colitis. *Mucosal Immunol* (2012) **5**:184–93. doi:10.1038/mi.2011.64
108. Goto Y, Panea C, Nakato G, Cebula A, Lee C, Diez MG, et al. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity* (2014) **40**:594–607. doi:10.1016/j.immuni.2014.03.005
109. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* (2007) **204**:1757–64. doi:10.1084/jem.20070590
110. Manicassamy S, Ravindran R, Deng J, Oluoch H, Denning TL, Kasturi SP, et al. Toll-like receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. *Nat Med* (2009) **15**:401–9. doi:10.1038/nm.1925
111. Molenaar R, Knippenberg M, Goverse G, Olivier BJ, De Vos AF, O'Toole T, et al. Expression of retinaldehyde dehydrogenase enzymes in mucosal dendritic cells and gut-draining lymph node stromal cells is controlled by dietary vitamin A. *J Immunol* (2011) **186**:1934–42. doi:10.4049/jimmunol.1001672
112. Cherayil BJ. Indoleamine 2,3-dioxygenase in intestinal immunity and inflammation. *Inflamm Bowel Dis* (2009) **15**:1391–6. doi:10.1002/ibd.20910
113. Matteoli G, Mazzini E, Iliev ID, Mileti E, Fallarino F, Puccetti P, et al. Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* (2010) **59**:595–604. doi:10.1136/gut.2009.185108
114. Laffont S, Siddiqui KR, and Powrie F. (2010). Intestinal inflammation abrogates the tolerogenic properties of MLN CD103+ dendritic cells. *Eur J Immunol* **40**:1877–1883. doi:10.1002/eji.200939957
115. Kinnebrew MA, Buffie CG, Diehl GE, Zenewicz LA, Leiner I, Hohl TM, et al. Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. *Immunity* (2012) **36**:276–87. doi:10.1016/j.immuni.2011.12.011
116. Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science* (2014) **343**:776–9. doi:10.1126/science.1247651
117. Zeisel A, Munoz-Manchado AB, Codeluppi S, Lonnerberg P, La Manno G, Jureus A, et al. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science* (2015) **347**:1138–42. doi:10.1126/science.aaa1934
118. Gregori G, Rajwa B, Patsekin V, Jones J, Furuki M, Yamamoto M, et al. Hyperspectral cytometry. *Curr Top Microbiol Immunol* (2014) **377**:191–210. doi:10.1007/82_2013_359
119. Bendall SC, Nolan GP, Roederer M, Chattopadhyay PK. A deep profiler's guide to cytometry. *Trends Immunol* (2012) **33**:323–32. doi:10.1016/j.it.2012.02.010

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Microglia versus myeloid cell nomenclature during brain inflammation

Melanie Greter, Iva Lelios and Andrew Lewis Croxford*

Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland

As immune sentinels of the central nervous system (CNS), microglia not only respond rapidly to pathological conditions but also contribute to homeostasis in the healthy brain. In contrast to other populations of the myeloid lineage, adult microglia derive from primitive myeloid precursors that arise in the yolk sac early during embryonic development, after which they self-maintain locally and independently of blood-borne myeloid precursors. Under neuro-inflammatory conditions such as experimental autoimmune encephalomyelitis, circulating monocytes invade the CNS parenchyma where they further differentiate into macrophages or inflammatory dendritic cells. Often it is difficult to delineate resident microglia from infiltrating myeloid cells using currently known markers. Here, we will discuss the current means to reliably distinguish between these populations, and which recent advances have helped to make clear definitions between phenotypically similar, yet functionally diverse myeloid cell types.

Keywords: microglia, macrophage, monocyte, dendritic cell, CNS inflammation

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****Correspondence:***
Melanie Greter,
Institute of Experimental Immunology,
University of Zurich,
Winterthurerstrasse 190, Zurich 8057,
Switzerland
greter@immunology.uzh.ch

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Introduction

Most tissues are populated by incredibly diverse and abundant myeloid cells. By contrast, the central nervous system (CNS) harbors comparatively few myeloid cell subsets. This is likely due to the immune privilege and relative isolation enjoyed by the CNS compared to other non-lymphoid tissues such as the gut or the lung, which are continually confronted with foreign entities. In the steady state, the CNS houses several populations of myeloid cells with distinct localizations including perivascular, choroid plexus, and meningeal macrophages/dendritic cells (DCs) and microglia, which are the most abundant (1). Microglia are considered the resident macrophages of the brain given that they are the only myeloid cells present in the CNS parenchyma. Microglia perform both homeostatic and immune-related functions and constitute about 5–20% of all cells in the CNS (2). They use their "ramified" morphology to act as immune sentinels, extending specialized processes, and sampling the local environment for foreign bodies (3, 4). Numerous recent reports have unmasked additional functions for microglia other than being simply the brain's intrinsic immune system. For example, microglia are also critical for neuronal development, adult neurogenesis, learning-dependent synapse formation, and brain homeostasis (5–7). Microglia are classified as tissue resident macrophages but are clearly ontogenically distinct from other members of the mononuclear phagocyte system (MPS), which includes DCs, monocytes, and macrophages. Microglia originate from primitive macrophages that derive from erythro-myeloid precursors in the yolk sac (8–10). These primitive yolk sac macrophages colonize the developing brain in mice as early as embryonic day 9.5 (8). Throughout adult life microglia remain of embryonic origin in the healthy CNS and maintain themselves locally without any detectable contribution from circulating

myeloid progenitors including monocytes. Yolk sac macrophages and microglia precursors in the developing brain express high levels of the fractalkine receptor (CX_3CR1) and are positive for the integrin alpha M (Itgam, also know as CD11b; macrophage-1 antigen, Mac-1), F4/80, and the macrophage-colony stimulating factor receptor 1 (Csf-1R, CD115) similar to adult microglia as described below (8). Compared to adult microglia, however, microglia precursors are $CD45^{hi}$. The development of microglia is dependent on Csf-1R (CD115), the transcription factors PU.1 and Irf8 but is independent of Myb, which is crucial for the development of hematopoietic stem cells (HSCs) (8, 9, 11, 12). In contrast to microglia, recent adoptive transfer and fate-mapping studies revealed that other macrophage populations are either embryonically derived from definitive hematopoiesis (e.g., alveolar or heart macrophages) or are constantly replaced by circulating monocytes (e.g., dermal or gut macrophages) (10, 13–18). Aside from the unique ontogeny of microglia within the MPS, a clear classification of microglia compared to other tissue macrophages in terms of phenotype and function has been difficult. Only recently, transcriptome and epigenetic analysis identified genes uniquely expressed and regulated by microglia but not by other macrophage populations (19–24). These studies might be useful to classify and distinguish microglia from other myeloid cells.

Microglia Markers in Steady State

In steady state conditions, microglia express surface markers typically present on many other tissue macrophages and/or monocytes such as CD11b, F4/80, Fc-gamma receptor 1 (CD64), and CD115 (Csf-1R), ionized calcium-binding adapter molecule 1 (Iba-1) and proto-oncogene tyrosine-protein kinase MER (MerTK) (**Figure 1**) (19). In contrast to microglia, which are γ-irradiation resistant, perivascular myeloid cells are replaced by bone marrow (BM)-derived precursors after total-body irradiation and BM transplantation (25–28). However, the exact ontogeny of (non-microglia) myeloid cells associated with the CNS and whether they are also able to maintain themselves locally is, to date, not known (29). These perivascular cells are equipped to present antigen (varying levels of MHCII and CD11c). Whether they represent a homogeneous distinct population or a heterogeneous population of macrophages and/or DCs is not entirely resolved. In the past, a cell expressing F4/80 was deemed to be a macrophage, whereas a cell expressing CD11c was considered a DC. It is clear now that subsets of DCs can also express F4/80 and certain macrophage populations express CD11c. Upon Flt3L treatment, a $CD11c^{+}MHCII^{+}$ population in the meninges and choroid plexus expanded, which is indicative of the DC lineage whose development is dependent on Flt3L signaling (27, 28). In addition, a limited number of $CD11c^{+}$ myeloid cells were also described to be in a juxtavascular location in the CNS parenchyma (30). These cells might, however, represent *bona fide* microglia expressing CD11c in certain regions of the brain. Further studies are required to dissect the ontogeny and characterize these elusive myeloid cells associated with the CNS in the steady state.

As common to many other macrophage populations including microglia, most of the CNS-associated myeloid cells also express CD11b, CD115, Iba-1, and F4/80 (31). Therefore, apart from their location, the only available means to unequivocally distinguish microglia from other CNS-resident myeloid populations (CNS-associated macrophages/DCs) and circulating monocytes

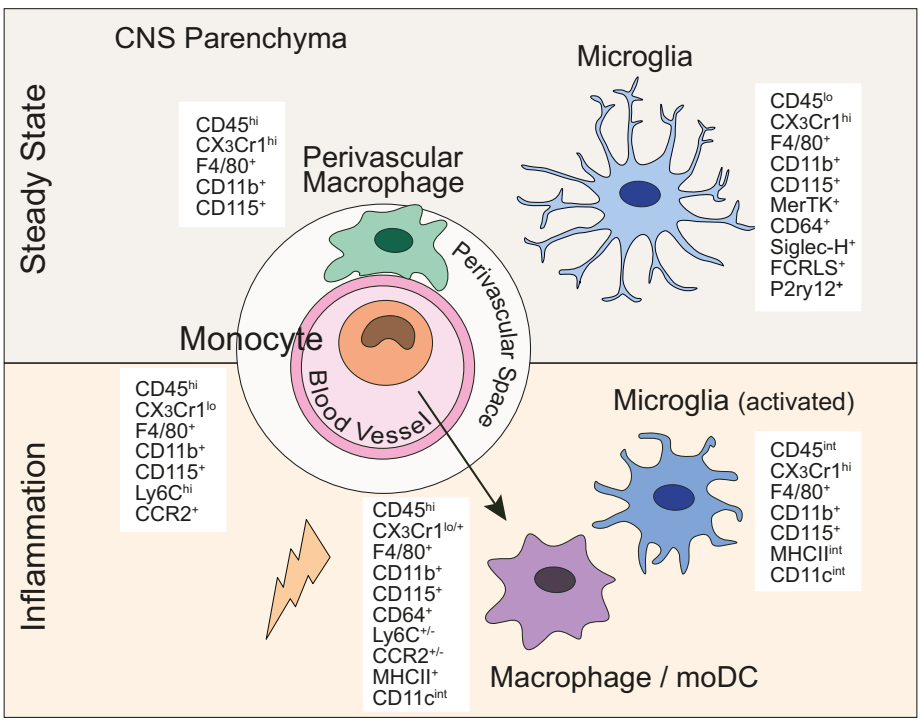

FIGURE 1 | Central nervous system myeloid cells and their defining lineage markers. In the steady state and under inflammatory conditions, myeloid cells in the CNS express a diverse, yet overlapping set of markers commonly used to discriminate between MPS members.

is by the reduced expression of the common leukocyte antigen CD45, which is readily detectable by flow cytometry. Adult microglia, unlike most other tissue macrophages, constitutively express high levels of the fractalkine receptor CX_3CR1 (32). A major advance in the microglia field has been the generation of *Cx3Cr1*^{creER} mice (32–34). This tamoxifen-inducible Cre-recombinase under the CX_3CR1 promoter allows for microglia-specific gene targeting. Despite the fractalkine receptor being expressed by monocytes and myeloid precursors in the BM, microglia remain a self-contained population in the CNS and therefore remain targeted long after ceasing of tamoxifen administration, returning the short-lived, circulating myeloid cells to their wild-type origin.

Only recently, gene expression studies have identified surface markers and transcription factors specifically expressed by steady state microglia but not by other macrophage populations or monocytes. These include, for example, sialic acid-binding immunoglobulin-type lectin H (Siglec-H), Fc receptor-like S (Fcrls), and purinergic receptor P2Y G-protein coupled 12 (P2ry12) (20, 21). Furthermore, microglia seem to be the only hematopoietic cell population that specifically expresses Sal-like 1 (Sall1), a transcription factor that plays a crucial role in kidney development (35). While previous studies have reported expression of Sall1 only by stromal cells, in the adult CNS this factor is expressed exclusively by microglia. These aforementioned gene expression studies have compared the transcriptome of microglia to either macrophages derived from the spleen, the lung, the peritoneum, or to monocytes. Whether these microglia core signature markers are also expressed by CNS-associated macrophages/DCs remains to be shown.

Microglia Markers in Inflammation

In contrast to the healthy brain, during neuro-inflammation the picture becomes far more complicated. A hallmark of microglia is their rapid activation after a CNS insult, resulting in their migration toward injury, proliferation, and their change in morphology. They take on a more "amoeboid" shape with shorter and thicker processes, display increased immunoreactivity for Iba-1 and upregulate CD45. Experimental autoimmune encephalomyelitis (EAE), which is a mouse model for multiple sclerosis (MS), is characterized by infiltration of T cells, monocytes, and neutrophils. Monocytes and their progeny [macrophages/monocyte-derived DCs (moDCs)] are undoubtedly the prevailing cell type in the lesions (see below). However, activated microglia are also clearly detected in the vicinity of the inflammatory lesions. The downregulation of Ly6C by monocytes upon their differentiation adds complexity to the separation of these two distinct cell types based on the commonly used cell surface markers. Additionally, molecules involved in antigen presentation and T cell stimulation, which are barely detectable in steady state microglia, are expressed to some level by microglia already at disease onset and retain expression throughout disease progression. These markers include major histocompatibility complex class II (MHCII), CD11c (also known as integrin alpha X, Itgax), CD80 (B7-1), CD86 (B7-2), and CD40 (36–38). Under these conditions, it is considerably more difficult to distinguish these activated microglia from inflammatory monocyte-derived cells. Similar changes in microglia surface markers have been observed in mouse models of neurodegenerative disease such as Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (ALS) (20, 31, 39).

A recent study, however, has used differentially expressed chemokine receptors on the surface of microglia and monocytes to distinguish those two myeloid populations and study their function during neuro-inflammation. Microglia were identified by their high expression of CX_3CR1 whereas infiltrating monocytes, which subsequently differentiate into macrophages/DCs, were defined by their high expression of C–C chemokine receptor type 2 (CCR2), a receptor mediating monocyte recruitment to sites of inflammation. Gene expression profiles from macrophages versus "embryonically derived" microglia at different stages of EAE show that despite some similarities between these inflammatory cell types, microglia exhibit a distinct molecular signature (40). This genetic distinction reflects a different function of resident microglia and infiltrating monocytes under pathological circumstances. While monocyte-derived macrophages seemed to be the effector cell type causing CNS damage, microglia might have a regulatory function and could play a role in tissue repair and homeostasis (40). Another report also showed that monocytes recruited to the CNS in EAE do not acquire microglia-signature genes (21). These studies will unquestionably help attributing unique functions to microglia and CNS-invading myeloid cells in different pathological conditions in the brain.

Whether microglia-specific surface markers and transcription factors alter their expression between steady state and inflammation remains unclear. The microglia-specific ATP receptor P2ry12 was downregulated under inflammatory conditions such as lipopolysaccharide (LPS) systemic injection or in SOD1 mouse-model of ALS (41, 42). On the other hand, P2ry12 and Fcrls continue to be expressed by microglia in EAE but are not expressed by infiltrating monocytes (21). Therefore, further studies need to be undertaken in order to better characterize these "new" phenotypic microglia markers under pathological conditions.

Monocyte-Derived Microglia

Even though microglia homeostasis is maintained through local self-renewal, under certain conditions circulating precursors can give rise to microglia-like cells. For example, early studies using BM-chimeras showed that up to 10–20% of microglia were reconstituted by donor-derived cells 6-12 months after total-body irradiation and BM transplantation (43). However, this engraftment of BM-derived microglia can only be seen upon blood–brain-barrier (BBB) disruption (e.g. irradiation) and becomes minimal in models where the BBB is unperturbed (e.g. protection of the head during irradiation and parabiosis) (44, 45). This clearly indicates that under steady state conditions, monocytes or BM-derived myeloid progenitors do not infiltrate the CNS parenchyma and thus do not give rise to adult microglia. Similarly, as described above in experimental models of neuro-inflammation, monocytes infiltrate the CNS and differentiate into effector cells resembling phenotypically activated microglia. Despite these similarities, monocyte-derived cells do not persist

in the CNS after inflammation has been resolved and thus do not contribute to long-lived microglia (46).

Finally, local administration of ganciclovir to transgenic mice expressing the thymidine kinase of herpes simplex virus under the CD11b promoter (CD11b-HSVTK) leads to a rapid depletion of microglia (47, 48). Subsequently, BM-derived cells enter the CNS and differentiate into long-lived microglia-like cells. Notably, while these cells form a network filling the niche for embryonically derived microglia, they do not obtain a complete microglia phenotype. Monocyte-derived "microglia" in this model show a less-ramified morphology and a higher expression of CD45 compared to yolk sac-derived microglia resembling more activated microglia (48). It has not yet been investigated whether these monocyte-derived "microglia" functionally resemble embryonically derived microglia or whether they acquire microglia-signature genes (Siglec-H, Fcrls, P2ry12) as described above.

Recent studies used a Csf-1R inhibitor to deplete microglia. Upon treatment stop, microglia were repopulated within 1 week (49, 50). These studies showed that new "microglia" were derived from CNS-resident nestin-positive precursors and resembled embryonically derived microglia in response to an inflammatory stimulus. Animals with newly repopulated "microglia" did not display any impairment in behavior, cognition, or motor function compared to control animals (50).

Monocytes and Monocyte-Derived Cells in CNS Inflammation

Brain inflammation or "encephalitis" invariably results in a reshaping of the myeloid cell populations inhabiting the CNS. An inflammatory response brought on by either infection or autoimmune manifestations results in a rapid increase in blood-derived cellularity to this otherwise dormant site. Despite EAE being fully dependent on T helper cells (51), the vast majority of the inflammatory infiltrate seen in EAE is of myeloid derivation. Two types of monocytes exist including the classical monocytes ($Ly6C^{hi}CCR2^{+}CX_3CR1^{lo}$) and the non-classical monocytes ($Ly6C^{lo}CCR2^{-}CX_3CR1^{hi}$). Here, we will only discuss $Ly6C^{hi}$ monocytes given that during neuro-inflammation, this is the subset recruited to the brain. Engraftment of phagocytes derived from circulating $CCR2^{+}$ monocytes has also been shown in an AD mouse model (39). $Ly6C^{hi}$ monocytes egress from the BM and cross the BBB in a CCR2-dependent manner (52, 53) followed by their differentiation into macrophages/moDCs and upregulation of a set of cell surface markers (e.g., MHCII, CD11c) expressed on a wide variety of MPS members. Likewise, microglia progressively alter their phenotype to resemble more classically activated macrophages during CNS inflammation, infection, and neuronal or myelin damage (54).

$Ly6C^{+}$ monocytes were shown to migrate into the CNS prior to disease onset and precede the development of paralysis and subsequent clinical manifestations of EAE, when "DC-like" cells are found in abundance in the inflamed tissue (55, 56). This corresponds well with a previous report showing $CD205^{+}$ myeloid cells accumulating in the meninges, choroid plexus, and subpial space of the spinal cord and in perivascular cuffs in demyelinating lesions during acute disease (57). $CD11b^{+}$ DCs within the inflamed CNS were demonstrated to be critical for the propagation of EAE (27, 58, 59). Further phenotypical characterization would be required to demarcate their lineage whether they resemble moDCs or are more similar to classical DCs. Indeed, monocyte-derived antigen presenting cells (APCs) have been shown to be required for optimal priming of T cells in models of infection (60). Current evidence suggests that phenotypically similar macrophages in the CNS can not only contribute to the generation of inflammatory lesions and perform a pathogenic role in the demyelination process but also contribute to regenerative repair mechanisms to resolve inflammation (61, 62). These studies emphasize that distinct functions are attributed to the different subsets of myeloid cells in the course of a CNS inflammation. As such, a complete understanding of cell types based on surface phenotype alone would be of great benefit both in preclinical models of CNS inflammation and also in human patients.

Even with the knowledge we now possess on myeloid cell diversity, it is still commonplace in the literature using animal models of CNS inflammation to use a simplistic $CD45^{hi}CD11b^{hi}$ gating strategy to separate CNS infiltrating, blood-derived myeloid cells from CNS-resident, embryonically derived microglia ($CD45^{low}$) (63). Efforts to sort cells using a broad $CD45^{hi}CD11b^{hi}$ surface phenotype from within the inflamed CNS will inevitably result in analysis of multiple cell types, lacking any of the desired specificity. Indeed, without the removal of $Ly6G^{hi}$ cells during sorting, a mixed population is inevitable and expression profiles subsequently attributed to moDCs are either confused with, or heavily influenced by, an abundant neutrophil contamination. Even if the effort is taken to remove neutrophils, moDCs at various stages of development will be incorporated. This distinction is increasingly important given that both neutrophils and moDCs have been shown to mediate BBB permeability and demyelination, and that different pathogenic mechanisms are likely active in the two populations during the same inflammation (40, 64).

We know that at least four clearly distinct cell types share this rather non-specific $CD45^{hi}CD11b^{hi}$ surface phenotype in an inflamed CNS, namely neutrophils ($CD11b^{+}Ly6G^{+}$), monocytes ($CD11b^{+}Ly6C^{hi}CX_3CR1^{low}$), and their progeny such as moDCs and/or activated macrophages (**Figure 1**). The latter two cell types represent most likely the same population with just different names assigned by different studies. $Ly6C^{hi}$ monocytes that have migrated into the CNS can further be subdivided into numerous differentiation stages characterized by the upregulation of CD11c and MHCII, with the concomitant downregulation of Ly6C and CCR2. Upon differentiation and upregulation of MHCII, monocytes are then called moDCs/activated macrophages. Thus, moDCs in the CNS are characterized by the expression of $CD11b^{+}F4/80^{+}MHCII^{+}CD11c^{int}Ly6C^{+/-}$. These moDCs/activated macrophages also express CD64 and likely also MerTK, which both are universally expressed by tissue macrophages including microglia (19). Interestingly, it has been shown that monocytes recruited to the CNS during EAE do not express the newly identified microglia markers Fcrls and P2ry12 highlighting again the diverse ontogeny of these cell types

and suggesting that the microglia-signature genes are indeed specific to microglia rather than location (CNS) specific (21). The FcεRIα (MAR-1) has been suggested to represent a moDC marker. Whether moDCs in the inflamed CNS express MAR-1 has so far not been analyzed (65). Perhaps a more functional distinction should be drawn on the level of relevance for the inflammatory process to persist. CNS-infiltrating myeloid cells with DC-like morphology express MHCII, CD40, and CD86, all of which have critical roles in multiple inflammatory models (66). The CD86/CD28 interaction between T cells and APCs is of critical importance for T cell activation. Furthermore, the CD40/CD40L interaction induces a maturation pathway within the inflamed CNS, resulting in further costimulatory capabilities and proinflammatory cytokine expression (67, 68). The levels of CD40 on monocyte-derived cells in the inflamed CNS are variable but generally not as high as on classical DCs.

After activation, inflammatory macrophages can not only express a wide range of inflammatory cytokines but also oxygen-based chemically reactive molecules involved in host defense. The route an activated macrophage takes depends largely on the T cell and/or NK cell-derived cytokines present during their activation. For example, activation in the presence of LPS and IFN-γ leads to a "classical" activation (often called "M1"), resulting in secretion of high levels of TNF-α, iNOS, IL-1, IL-6, and IL-12. Conversely, activation of the same cells in the presence of IL-4 and IL-10 will result in rapid upregulation of IL-10, production of Arginase-1, and upregulation of the mannose receptor CD206, generating a macrophage capable of suppressing T cell activity (called "M2") (69). This intracellular divergence in phenotype illustrates that an apparently similar cell expressing F4/80, CD64, CD11b, MHCII, and CD11c on its surface may, in fact, differ greatly in its function. Indeed, markers identifying both M1 and M2 macrophage populations have been shown synergistically in CNS biopsies obtained from MS patients. CD40, CD64, CD86, and CD32, mannose receptor and CD163 were co-expressed in the large majority of foamy macrophages found in lesional CNS (70). Therefore, surface characterization of inflammatory macrophages would appear insufficient and may mask different macrophage populations in direct opposition to each other, depending on the type of inflammation taking place. Generally in the steady state, tissue macrophages display an "M2-like" phenotype and are critical for tissue homeostasis. Interestingly, in a model of spinal cord injury, it was shown that M2 macrophages ($CD11b^{+}F4/80^{+}CX_3CR1^{hi}Ly6C^{lo}$) are beneficial and promote recovery (62).

Conclusion

Under steady state conditions, site specific and phenotypic characteristics exist to distinguish between microglia and other CNS-associated macrophages. As with almost all innate and adaptive immune cell types, consensus with respect to nomenclature in CNS-resident versus CNS-infiltrating myeloid cells has not been effectively reached under inflammatory conditions. The advent of microarray technology and next generation sequencing will serve to provide more useful ways to distinguish between these two apparently similar, yet ever more functionally diverse cell types. An ever-increasing variety of previously unappreciated, and non-immune homeostatic functions performed by macrophages are now beginning to emerge, making a more detailed separation of these cell types highly desirable (71). Ultimately, better characterization and dissection of the various myeloid cells in an inflamed brain will help deciphering the specialized functions of the different members of the MPS in pathological conditions.

References

1. Katsumoto A, Lu H, Miranda AS, Ransohoff RM. Ontogeny and functions of central nervous system macrophages. *J Immunol* (2014) **193**:2615–21. doi:10.4049/jimmunol.1400716
2. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* (1990) **39**:151–70. doi:10.1016/0306-4522(90)90229-W
3. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* (2005) **8**:752–8. doi:10.1038/nn1472
4. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* (2005) **308**:1314–8. doi:10.1126/science.1110647
5. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science* (2011) **333**:1456–8. doi:10.1126/science.1202529
6. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* (2012) **74**:691–705. doi:10.1016/j.neuron.2012.03.026
7. Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G, Pagani F, et al. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nat Neurosci* (2014) **17**:400–6. doi:10.1038/nn.3641
8. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) **330**:841–5. doi:10.1126/science.1194637
9. Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat Neurosci* (2013) **16**:273–80. doi:10.1038/nn.3318
10. Perdiguero EG, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2014) **518**(7540):547–51. doi:10.1038/nature13989
11. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**:86–90. doi:10.1126/science.1219179
12. Shiau CE, Kaufman Z, Meireles AM, Talbot WS. Differential requirement for irf8 in formation of embryonic and adult macrophages in zebrafish. *PLoS One* (2015) **10**:e0117513. doi:10.1371/journal.pone.0117513
13. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* (2007) **204**:171–80. doi:10.1084/jem.20061011
14. Bogunovic M, Ginhoux F, Helft J, Shang L, Hashimoto D, Greter M, et al. Origin of the lamina propria dendritic cell network. *Immunity* (2009) **31**:513–25. doi:10.1016/j.immuni.2009.08.010
15. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* (2013) **210**:1977–92. doi:10.1084/jem.20131199
16. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) **15**:929–37. doi:10.1038/ni.2967
17. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained

through distinct mechanisms at steady state and during inflammation. *Immunity* (2014) **40**:91–104. doi:10.1016/j.immuni.2013.11.019
18. Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, et al. C-myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* (2015) **42**:665–78. doi:10.1016/j.immuni.2015.03.011
19. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**:1118–28. doi:10.1038/ni.2419
20. Chiu IM, Morimoto ET, Goodarzi H, Liao JT, O'Keeffe S, Phatnani HP, et al. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep* (2013) **4**:385–401. doi:10.1016/j.celrep.2013.06.018
21. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. *Nat Neurosci* (2014) **17**:131–43. doi:10.1038/nn.3599
22. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**:1327–40. doi:10.1016/j.cell.2014.11.023
23. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
24. Zeisel A, Munoz-Manchado AB, Codeluppi S, Lonnerberg P, La Manno G, Jureus A, et al. Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science* (2015) **347**:1138–42. doi:10.1126/science.aaa1934
25. Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* (1988) **239**:290–2. doi:10.1126/science.3276004
26. Lassmann H, Hickey WF. Radiation bone marrow chimeras as a tool to study microglia turnover in normal brain and inflammation. *Clin Neuropathol* (1993) **12**:284–5.
27. Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, et al. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat Med* (2005) **11**:328–34. doi:10.1038/nm1197
28. Anandasabapathy N, Victora GD, Meredith M, Feder R, Dong B, Kluger C, et al. Flt3L controls the development of radiosensitive dendritic cells in the meninges and choroid plexus of the steady-state mouse brain. *J Exp Med* (2011) **208**:1695–705. doi:10.1084/jem.20102657
29. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**:792–804. doi:10.1016/j.immuni.2013.04.004
30. Prodinger C, Bunse J, Kruger M, Schiefenhovel F, Brandt C, Laman JD, et al. CD11c-expressing cells reside in the juxtavascular parenchyma and extend processes into the glia limitans of the mouse nervous system. *Acta Neuropathol* (2011) **121**:445–58. doi:10.1007/s00401-010-0774-y
31. Prinz M, Priller J, Sisodia SS, Ransohoff RM. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat Neurosci* (2011) **14**:1227–35. doi:10.1038/nn.2923
32. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**:79–91. doi:10.1016/j.immuni.2012.12.001
33. Goldmann T, Wieghofer P, Muller PF, Wolf Y, Varol D, Yona S, et al. A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. *Nat Neurosci* (2013) **16**:1618–26. doi:10.1038/nn.3531
34. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR III, Lafaille JJ, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* (2013) **155**:1596–609. doi:10.1016/j.cell.2013.11.030
35. Nishinakamura R, Matsumoto Y, Nakao K, Nakamura K, Sato A, Copeland NG, et al. Murine homolog of SALL1 is essential for ureteric bud invasion in kidney development. *Development* (2001) **128**:3105–15.
36. Juedes AE, Ruddle NH. Resident and infiltrating central nervous system APCs regulate the emergence and resolution of experimental autoimmune encephalomyelitis. *J Immunol* (2001) **166**:5168–75. doi:10.4049/jimmunol.166.8.5168
37. Ponomarev ED, Shriver LP, Maresz K, Dittel BN. Microglial cell activation and proliferation precedes the onset of CNS autoimmunity. *J Neurosci Res* (2005) **81**:374–89. doi:10.1002/jnr.20488
38. Almolda B, Gonzalez B, Castellano B. Antigen presentation in EAE: role of microglia, macrophages and dendritic cells. *Front Biosci* (2011) **16**:1157–71. doi:10.2741/3781
39. Mildner A, Schlevogt B, Kierdorf K, Bottcher C, Erny D, Kummer MP, et al. Distinct and non-redundant roles of microglia and myeloid subsets in mouse models of Alzheimer's disease. *J Neurosci* (2011) **31**:11159–71. doi:10.1523/JNEUROSCI.6209-10.2011
40. Yamasaki R, Lu H, Butovsky O, Ohno N, Rietsch AM, Cialic R, et al. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med* (2014) **211**:1533–49. doi:10.1084/jem.20132477
41. Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, et al. The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat Neurosci* (2006) **9**:1512–9. doi:10.1038/nn1805
42. Butovsky O, Jedrychowski MP, Cialic R, Krasemann S, Murugaiyan G, Fanek Z, et al. Targeting miR-155 restores abnormal microglia and attenuates disease in SOD1 mice. *Ann Neurol* (2015) **77**:75–99. doi:10.1002/ana.24304
43. Kennedy DW, Abkowitz JL. Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. *Blood* (1997) **90**(3):986–93.
44. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* (2007) **10**:1538–43. doi:10.1038/nn2014
45. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, et al. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci* (2007) **10**:1544–53. doi:10.1038/nn2015
46. Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci* (2011) **14**:1142–9. doi:10.1038/nn.2887
47. Heppner FL, Greter M, Marino D, Falsig J, Raivich G, Hovelmeyer N, et al. Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nat Med* (2005) **11**:146–52. doi:10.1038/nm0405-455
48. Varvel NH, Grathwohl SA, Baumann F, Liebig C, Bosch A, Brawek B, et al. Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. *Proc Natl Acad Sci U S A* (2012) **109**:18150–5. doi:10.1073/pnas.1210150109
49. Elmore MR, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA, et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron* (2014) **82**:380–97. doi:10.1016/j.neuron.2014.02.040
50. Elmore MR, Lee RJ, West BL, Green KN. Characterizing newly repopulated microglia in the adult mouse: impacts on animal behavior, cell morphology, and neuroinflammation. *PLoS One* (2015) **10**:e0122912. doi:10.1371/journal.pone.0122912
51. Schreiner B, Heppner FL, Becher B. Modeling multiple sclerosis in laboratory animals. *Semin Immunopathol* (2009) **31**:479–95. doi:10.1007/s00281-009-0181-4
52. Fife BT, Huffnagle GB, Kuziel WA, Karpus WJ. CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *J Exp Med* (2000) **192**:899–905. doi:10.1084/jem.192.6.899
53. Gaupp S, Pitt D, Kuziel WA, Cannella B, Raine CS. Experimental autoimmune encephalomyelitis (EAE) in CCR2(-/-) mice: susceptibility in multiple strains. *Am J Pathol* (2003) **162**:139–50. doi:10.1016/S0002-9440(10)63805-9
54. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci* (1996) **19**:312–8. doi:10.1016/0166-2236(96)10049-7
55. King IL, Dickendesher TL, Segal BM. Circulating Ly-6C+ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. *Blood* (2009) **113**:3190–7. doi:10.1182/blood-2008-07-168575
56. Mildner A, Mack M, Schmidt H, Bruck W, Djukic M, Zabel MD, et al. CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system. *Brain* (2009) **132**:2487–500. doi:10.1093/brain/awp144
57. Serafini B, Columba-Cabezas S, Di Rosa F, Aloisi F. Intracerebral recruitment and maturation of dendritic cells in the onset and progression of experimental autoimmune encephalomyelitis. *Am J Pathol* (2000) **157**:1991–2002. doi:10.1016/S0002-9440(10)64838-9

58. McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat Med* (2005) **11**:335–9. doi:10.1038/nm1202
59. Bailey SL, Schreiner B, McMahon EJ, Miller SD. CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4+ T(H)-17 cells in relapsing EAE. *Nat Immunol* (2007) **8**:172–80. doi:10.1038/ni1430
60. Schreiber HA, Loschko J, Karssemeijer RA, Escolano A, Meredith MM, Mucida D, et al. Intestinal monocytes and macrophages are required for T cell polarization in response to *Citrobacter rodentium*. *J Exp Med* (2013) **210**:2025–39. doi:10.1084/jem.20130903
61. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci* (2009) **29**:13435–44. doi:10.1523/JNEUROSCI.3257-09.2009
62. Shechter R, Miller O, Yovel G, Rosenzweig N, London A, Ruckh J, et al. Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. *Immunity* (2013) **38**:555–69. doi:10.1016/j.immuni.2013.02.012
63. Vainchtein ID, Vinet J, Brouwer N, Brendecke S, Biagini G, Biber K, et al. In acute experimental autoimmune encephalomyelitis, infiltrating macrophages are immune activated, whereas microglia remain immune suppressed. *Glia* (2014) **62**:1724–35. doi:10.1002/glia.22711
64. Aube B, Levesque SA, Pare A, Chamma E, Kebir H, Gorina R, et al. Neutrophils mediate blood-spinal cord barrier disruption in demyelinating neuroinflammatory diseases. *J Immunol* (2014) **193**:2438–54. doi:10.4049/jimmunol.1400401
65. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* (2013) **38**:322–35. doi:10.1016/j.immuni.2012.10.016
66. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* (1998) **392**:245–52. doi:10.1038/32588
67. Stout RD, Suttles J, Xu J, Grewal IS, Flavell RA. Impaired T cell-mediated macrophage activation in CD40 ligand-deficient mice. *J Immunol* (1996) **156**:8–11.
68. Becher B, Durell BG, Miga AV, Hickey WF, Noelle RJ. The clinical course of experimental autoimmune encephalomyelitis and inflammation is controlled by the expression of CD40 within the central nervous system. *J Exp Med* (2001) **193**:967–74. doi:10.1084/jem.193.8.967
69. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* (2008) **8**:958–69. doi:10.1038/nri2448
70. Vogel DY, Vereyken EJ, Glim JE, Heijnen PD, Moeton M, Van Der Valk P, et al. Macrophages in inflammatory multiple sclerosis lesions have an intermediate activation status. *J Neuroinflammation* (2013) **10**:35. doi:10.1186/1742-2094-10-35
71. Muller PA, Koscso B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* (2014) **158**:300–13. doi:10.1016/j.cell.2014.04.050

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REVIEW
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Revisiting mouse peritoneal macrophages: heterogeneity, development, and function

Alexandra dos Anjos Cassado[1], Maria Regina D'Império Lima[1] and Karina Ramalho Bortoluci[2,3]*

[1] Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil, [2] Centro de Terapia Celular e Molecular (CTC-Mol), Universidade Federal de São Paulo, São Paulo, Brazil, [3] Departamento de Ciências Biológicas, Campus Diadema, Universidade Federal de São Paulo, São Paulo, Brazil

Tissue macrophages play a crucial role in the maintenance of tissue homeostasis and also contribute to inflammatory and reparatory responses during pathogenic infection and tissue injury. The high heterogeneity of these macrophages is consistent with their adaptation to distinct tissue environments and specialization to develop niche-specific functions. Although peritoneal macrophages are one of the best-studied macrophage populations, recently it was demonstrated the co-existence of two subsets in mouse peritoneal cavity (PerC), which exhibit distinct phenotypes, functions, and origins. These macrophage subsets have been classified, according to their morphology, as large peritoneal macrophages (LPMs) and small peritoneal macrophages (SPMs). LPMs, the most abundant subset under steady state conditions, express high levels of F4/80 and low levels of class II molecules of the major histocompatibility complex (MHC). LPMs appear to be originated from embryogenic precursors, and their maintenance in PerC is regulated by expression of specific transcription factors and tissue-derived signals. Conversely, SPMs, a minor subset in unstimulated PerC, have a F4/80^{low}MHC-II^{high} phenotype and are generated from bone-marrow-derived myeloid precursors. In response to infectious or inflammatory stimuli, the cellular composition of PerC is dramatically altered, where LPMs disappear and SPMs become the prevalent population together with their precursor, the inflammatory monocyte. SPMs appear to be the major source of inflammatory mediators in PerC during infection, whereas LPMs contribute for gut-associated lymphoid tissue-independent and retinoic acid-dependent IgA production by peritoneal B-1 cells. In the previous years, considerable efforts have been made to broaden our understanding of LPM and SPM origin, transcriptional regulation, and functional profile. This review addresses these issues, focusing on the impact of tissue-derived signals and external stimulation in the complex dynamics of peritoneal macrophage populations.

Keywords: peritoneal macrophages, peritoneal cavity, LPM, SPM, origin

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****Correspondence:***
Alexandra dos Anjos Cassado, Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Professor Lineu Prestes, 1730, Cidade Universitária, São Paulo, SP 05508-900, Brazil
alecassado@hotmail.com

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Introduction

Macrophages are resident cells found in almost all tissues of the body, where they assume specific phenotypes and develop distinct functions. Tissue macrophages are considered as immune sentinels because of their strategic localization and their ability to initiate and modulate immune responses

during pathogenic infection or tissue injury and to contribute to the maintenance of tissue homeostasis (1–3). Macrophages were first identified in the late 19th century by Élie Metchnikoff (1845–1916) and designated as large phagocytes (4, 5). Based on their phagocytic activity, macrophages were first classified as cells from the reticuloendothelial system, which also comprised endothelial cells, fibroblasts, spleen and lymphoid reticular cells, Kupffer cells, splenocytes, and monocytes (6). However, because endocytosis performed by endothelial cells is a process that is distinct from phagocytosis, by the late 1960s a new classification system for mononuclear phagocytic cells as cells from "mononuclear phagocytic system" (MPS) was proposed (7). The MPS was defined as a group of phagocytic cells sharing morphological and functional similarities, including pro-monocytes, monocytes, macrophages, dendritic cells (DCs), and their bone marrow (BM) progenitors (7–12). Although the phagocytic cells play similar roles in orchestrating the immune response and maintaining tissue homeostasis (11), they represent cell populations that are extremely heterogeneous (13), and the general classification of mononuclear cells in a unique system is currently under intense discussion (12, 14). In this context, Guilliams et al. suggested a classification of MPS cells based primarily on their ontogeny and secondary on their location, function, and phenotype, promoting a better classification under both steady state and inflammatory conditions (14).

In the last few years, a complex scenario to describe macrophage origins has been developed (15–19), replacing the simplistic view of myeloid precursors giving rise to blood monocytes that, in turn, originate tissue macrophages (20–22). For example, resident macrophages from brain, lung, liver, peritoneum, and spleen are not differentiated from monocytes; instead, they are derived from an embryonic precursor and maintained by self-renewal (23–27). In addition to resident macrophages, infiltrating monocytes are also found in injured tissues, where they can differentiate into inflammatory macrophages or TNF-α- and inducible nitric oxide synthase (iNOS)-producing (Tip)-DCs (28). Currently, it is accepted that inflammatory macrophages and tissue-resident macrophages comprise developmentally and functionally distinct populations (3, 14, 17, 18, 29).

Under steady state conditions, some tissues and serous cavities, including lung, spleen, and the peritoneal cavity (PerC), present distinct resident macrophage subpopulations. In the spleen, at least three macrophage subsets are found: red pulp, metalophilic, and marginal zone macrophages (30). In the PerC, two peritoneal macrophage subsets have been described: large peritoneal macrophage (LPM) and small peritoneal macrophage (SPM) (31). Mouse peritoneal macrophages are among the best-studied macrophage populations in terms of cell biology, development, and inflammatory responses (24, 31–42). Peritoneal macrophages play key roles in the control of infections and inflammatory pathologies (43, 44), as well as in the maintenance of immune response robustness (40). Therefore, this review will discuss recent advances in our understanding of peritoneal macrophage subsets characterization, origin and functions, and the accurate experimental approaches to analyze them.

Identification of Peritoneal Macrophages

Cohn and collaborators introduced the study of peritoneal macrophages (45–48). Indeed, a representative portion of the current knowledge regarding macrophage biology, such as their function, specialization, and development stems from studies performed using peritoneal macrophages as a cellular source. However, the existence of two resident macrophage subsets present in the PerC was described recently (31). These macrophage subsets were designated LPM and SPM according to their size. LPMs and SPMs were initially identified based on their differential expression of F4/80 and CD11b, where LPMs express high levels of F4/80 and CD11b while SPMs show F4/80^{low}CD11b^{low} phenotype (**Table 1**). CD11b is an integrin that, together with CD18, forms the CR3 heterodimer (13, 30, 49), but is not exclusively expressed on macrophages and is found on several others cell types, including polymorphonuclear cells (50, 51), DCs (52), and at low levels on B lymphocytes (53, 54). F4/80, a 160 kD glycoprotein from the epidermal growth factor (EGF)-transmembrane 7 (TM7) family, is expressed by macrophages in several organs, such as the kidney (55), BM (56), epithelium (57), lung (58, 59), lymphoid organs (60), and among others (61, 62), and it is not found on fibroblasts, polymorphonuclear cells, and lymphocytes (63). However, peritoneal eosinophils show low levels of F4/80 (31) and some macrophage subpopulations exhibit low levels or do not express F4/80, such as white pulp and marginal zone splenic macrophages (30). Therefore, F4/80 expression levels distinguish macrophage subpopulations, including those residing in the same tissue, such as subsets found in the spleen and PerC (30, 31, 35). In this sense, the great majority (approximately 90%) of F4/80^{+}CD11b^{+} cells present in the PerC from several mouse strains, including BALB/c, C57BL/6, 129/S6, FVB/N, SJL/J, and RAG$^{-/-}$, express high levels of these molecules and correspond to the LPM subset, whereas the minor SPM subset expresses low levels of these markers (31).

An accurate evaluation of SPMs and LPMs by flow cytometry and optical microscopy revealed that in addition to the differential expression of CD11b and F4/80, SPMs and LPMs display unique morphologies and phenotypes. LPMs assume the

TABLE 1 | Phenotypic profile of SPMs and LPMs.

Surface molecule	LPMs	SPMs
F4/80	+++	+
CD11b	+++	+
CD11c	+	−
MHC-II	+	++
GR1	+	−
Ly6C	−	−
c-kit	−	−
CD62L	−	++
Dectin-1	+	++
DC-Sign	−	++
TLR4	++	+
CD80	++	+
CD86	+++	+
CD40	++	+
12/15-LOX	+	−
TIM4	+	−

classical morphology described for macrophages after adherence, exhibiting prominent vacuolization and abundant cytoplasm, whereas SPMs display a polarized morphology in culture, presenting dendrites similar to DCs (35). Moreover, the analysis of a complex panel of cell surface molecules (**Table 1**) demonstrated that SPMs express higher levels of MHC-II (IA^b), dectin-1, and DC-sign endocytic receptors than LPMs. Moreover, half of SPM subset expresses high levels CD62L (31, 35, 36). Conversely, LPMs express higher levels of toll like receptor (TLR)-4 and co-stimulatory molecules in comparison to SPMs (31, 35, 36).

Given that PerC is a singular compartment where specialized immune cells reside and interact, including macrophages, B cells, DCs, eosinophils, mast cells, neutrophils, T cells, natural killer (NK), and invariant NKT cells (31, 32, 35, 36, 64), the identification of myeloid cells from PerC based on cell surface molecules is still a complex matter, particularly in terms of distinguishing macrophage subsets from DCs and inflammatory monocytes. The expression of 12/15-lipoxygenase (LOX), Tim4, and Ly6B has also been examined to discriminate heterogeneous macrophage subsets in PerC under steady state conditions and during peritonitis (24, 37, 38, 42). The high expression of 12/15-LOX and Tim4 was observed in peritoneal macrophages, which also express high levels of F4/80 and CD11b, correlating with the phenotype and frequencies observed for LPMs (24, 31, 37, 38, 42). Conversely, 12/15-LOX^- cells and SPM share the same $CD11b^+F4/80^{low}MHCII^{high}$ phenotype; however, 12/15-LOX^- cells express high levels of CD11c and co-stimulatory molecules, suggesting that 12/15-LOX^- cells and SPMs are, at least in part, distinct populations (31, 35, 37). Despite similarities in cell morphology and MHC-II expression presented by SPMs and DCs, the possibility that SPMs may be part of the peritoneal DC pool is excluded by the smaller size, the distinct and lack of the CD11b and F4/80 expression presented by DCs and, primarily, by the lower expression of CD11c (HL3 or N418 clones of monoclonal anti-CD11c) on SPMs compared with LPMs or typical peritoneal DCs (31, 35).

Given the cell complexity present in PerC and the importance of the development of efficient strategies to correctly identify macrophage subsets as well as to avoid contamination by other cell populations and misinterpretation of peritoneal macrophage studies, our group has proposed a simple way to identify peritoneal macrophage subsets using a four-color flow cytometry staining panel. From doublet, $CD19^{high}$ and $CD11c^{high}$ discarded selected cell populations; the analysis of $F4/80^+$ cells based on MHCII expression defines three distinct subpopulations, $F4/80^{high}IA^{b\text{-}neg}$, $F4/80^{low}IA^{b\text{-}high}$, and $F4/80^{low}IA^{b\text{-}neg}$, which correspond, respectively, to LPMs, SPMs, and granulocytes (35).

Origin and Development of LPM and SPM

The theories that explain the origin of macrophages have been completely reformulated in the last few years. The differentiation process of monocytes, macrophages, and DCs that occurs in the BM starts with the earliest progenitor, the hematopoietic stem cell (HSC), and follows the common myeloid progenitor (CMP) and the granulocyte and macrophage progenitor (GMP) (16). The clonotypic BM-resident precursor differentiated from GMP, termed the macrophage-DC precursor (MDP), expresses high levels of the fractalkine receptor CX3CR1, c-kit, and CD115, and gives rise to circulating blood monocytes, some macrophage populations and a common DC precursor (CDP), but does not originate granulocytes (15, 65, 66). The recruitment of monocyte subsets under steady state or inflammatory and pathological conditions depends on particular chemokines and the expression of their counterpart's receptors. The $Ly6C^+$ monocyte subset migrates via a CCR2-dependent pathway, whereas $Ly6C^-$ appears to migrate in response to CX3CR1 signaling (67). Under steady state conditions, extravasated monocytes do not contribute to the pool of resident macrophages in many tissues (3, 15, 16). In inflammatory settings, the $Ly6C^+$ monocyte subset differentiates into inflammatory macrophages and monocyte-derived DCs, such as Tip-DCs (15, 16).

Recent accumulating evidence supports the prenatal origin of tissue-resident macrophages and the idea that they are maintained locally by self-renewal throughout adult life, both in the steady state and after cell turnover, which is predominantly independent of hematopoiesis (17, 18, 23–27, 29, 68, 69). Microglia, Langerhans cells, Kupffer cells, red pulp splenic macrophages, lung, and peritoneal macrophages are originated from embryogenic precursor and proliferative cells maintained by self-renewal (23–27, 69–71). Fetal-liver monocytes or primitive macrophages found in the yolk sac, an extraembryonic tissue, have been related with the origin of tissue-resident macrophages. In this context, recent date using yolk sac macrophages depletion and fate-mapping models demonstrated that yolk sac macrophages, which are generated from early erythro-myeloid progenitors (EMPs), are important for development of macrophages in mid-gestation; however in adulthood, only microglia is maintained by these embryogenic precursor (69). In contrast, fetal monocytes that are derived from late EMPs give rise to tissue-resident macrophages from liver, lung, skin, kidney and spleen (69). The exception to the origin of resident macrophages is intestinal macrophages, which are continuously repopulated by circulating monocytes (72).

Understanding the dynamics of maintenance and recruitment of peritoneal macrophages is of particular interest since these cells are involved in physiological as well as pathological processes, such as peritonitis, tumors, and pancreatitis (40, 43, 44). Early studies demonstrated that peritoneal macrophages are maintained in PerC through self-renewal in the steady state or under inflammatory conditions (73–76). The omentum, a fat tissue that connects the abdominal organs, is also involved in peritoneal macrophage development through the proliferative capacities of omental macrophages (75, 76). The combination of these early observations, which were acquired recently, with the technical advances to correctly identify the peritoneal macrophage subsets has permitted the ontogeny of the peritoneal macrophage subsets to be elucidated (24, 31, 36, 39, 40, 42).

Under steady state conditions, LPMs appear to be maintained by self-renewal and independent of hematopoiesis (26, 36), whereas SPMs are originated from circulating monocytes (31, 36, 40) (**Figure 1**). Dates from Schulz et al. suggest that, in general, F4/80 expression by tissue macrophages correlated with yolk sac ($F4/80^{high}$) and not hematopoietic ($F4/80^{low}$) progenitors (25). In the $CX3CR1^{GFP/WT}$ mice, Cain et al. (36) showed the presence of

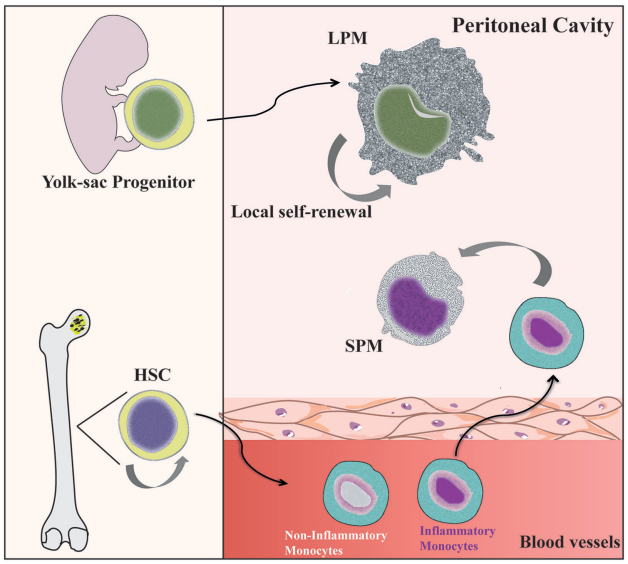

FIGURE 1 | Distinct origin of peritoneal macrophage subsets. SPMs are generated from hematopoietic stem cells (HSC) in the bone marrow (BM) by differentiation of inflammatory blood monocytes (31, 40). However, LPMs appear to be originated from progenitors from yolk sac and independent of hematopoietic progenitors (69). Local proliferation of LPMs ensures homeostatic maintenance by self-renewal (36).

GFP^+ cells in DC and SPM pool, but not in the LPM population. Conversely, in the $CX_3CR1CreRosa26R\text{-}FGFP$ mice, which show the active and past expression of CX3CR1, the presence of GFP^+ cells was found within DC, SPM, and LPM populations. These data indicate that SPMs are short-lived cells, whereas LPMs have a more distant ontogenic relationship with a $CX3CR1^+$ progenitor, corroborating the idea that they originate from the yolk sac (36). However, in chimeric C57BL/6 mice reconstituted with C57BL/6-CD45.1 BM, around 80% of SPMs and more than 70% of LPMs are CD45.1-expressing cells, demonstrating that both peritoneal macrophage subsets differentiate from BM precursors after ablation of peritoneal macrophages induced by irradiation (36). Data from our group suggest that PerC recruited $Ly6C^+$ monocytes could give rise to SPMs during inflammatory conditions (31). Confirming that SPMs are generated via the differentiation of inflammatory monocytes recruited to PerC, reduced numbers of SPMs are found in the PerC of $CCR2^{-/-}$ mice (40).

The analysis of Ki67 and phosphorylated histone H3 (pHH3 at a discrete stage of mitosis) staining and the quantification of cell cycle and basal DNA content revealed that the number of proliferating $F4/80^{high}CD11b^{high}$ cells decreases in 12-week-old mice compared with proliferation capacity of this population in newborn mice (15 days to 4 weeks) (24). After 12–16 weeks, the number of $F4/80^{high}CD11b^{high}$ cells in PerC is maintained under a low rate of proliferation, which suggests that the number of $F4/80^{high}CD11b^{high}$ peritoneal cells increases during mouse development until PerC acquires sufficient homeostatic cell numbers (24). Indeed, BrdU-labeled LPM frequencies after a single BrdU pulse were 7 and 15-fold lower than those found in HSC and GMP, respectively. Moreover, the presence of $BrdU^+$ LPMs was detectable 14 days after BrdU pulse, suggesting that they are a long-lived population, i.e., maintained at low levels of proliferation (36). Conversely, the detection of low numbers of proliferating SPMs at 6–10 days after one pulse of BrdU suggests that these cells have a low proliferation rate under steady state conditions and are short-lived cells (36).

Studies with mice deficient in CCAAT/enhancer binding protein (C/EBP)b also support the notion that LPMs and SPMs represent distinct ontogenies, because in the absence of this transcription factor, PerC did not contain LPMs and exhibited increased numbers of SPMs (36). Interestingly, adoptively transferred SPMs differentiated into LPMs in $Cebpb^{-/-}$ mice. However, in control mice that have normal numbers of LPMs, only a small frequency of transferred SPMs acquired the $F4/80^{hi}MHCII^{low}CD93^+$ phenotype of LPMs. Based on these results, the authors proposed that under physiological conditions, SPMs appear to contribute in only a small way to generate LPMs, but SPMs could be involved in the maintenance of LPMs in situations where this pool has been greatly reduced, such as under inflammatory conditions or following radiation ablation (36). These data are consistent with the findings of Yona et al. (26), which demonstrated the presence of monocyte-derived cells in the LPM compartment 8 weeks after the i.p. injection of thioglycollate. Together with LPMs, a subset of proliferating BM-derived inflammatory macrophage has also been associated with self-renewal mechanisms during the resolution of peritonitis induced by zymosan and thioglycollate (42). Conversely, LPMs do not seem to contribute to the SPM pool, even during inflammation. Our group demonstrated that adoptively transferred CFDA-SE-labeled LPMs 1 h after LPS stimulation retained its phenotype, and no $CFDA\text{-}SE^+$ cells were found in the SPM compartment until 2 days after stimulation (31).

In the last year, a great advance in the understanding of the transcriptional control of peritoneal macrophages provided novel insights into this scenario (39, 40). The zinc finger transcription factor GATA-binding protein 6 (GATA6) appears to regulate the majority of peritoneal macrophage-specific genes (PMSGs). Of note, GATA6 is selectively expressed by LPMs (40). Accordingly, the number of LPMs were greatly reduced in peritoneal lavages from $GATA6\text{-}KO^{mye}$ and Mac-GATA6 KO mice, which have a GATA6 deficiency in all myeloid cells or only in the macrophage lineages, respectively (39, 40). Interestingly, retinoic acid (RA) is the extracellular factor that regulates GATA-6-specific gene expression in LPMs, because vitamin A depleted (VAD; the RA precursor) mice exhibited a decrease in GATA6 expression and LPM numbers (40). Moreover, the stimulation of peritoneal macrophages from VAD mice with all-trans RA restored the expression of GATA-6 and many PMSGs at levels found in peritoneal macrophages from control mice. In addition to the regulation of gene expression profiling in peritoneal macrophages, GATA-6 appears to be involved in the control of the proliferation, survival, and metabolism of these cells (39, 77). GATA-6-deficient macrophages demonstrate an altered proliferation state during peritonitis (39). Moreover, $Lyz2\text{-}Cre \times GATA6^{(flox/flox)}$ mice also exhibit reduced numbers of peritoneal macrophages, which could be explained by the perturbation in their metabolism, culminating in the high frequency of cell death found in this compartment (77). Despite great contributions to our understanding in the

involvement of GATA-6 in peritoneal macrophage development, metabolism, self-maintenance, and survival, the existence of distinct pathways that could govern the transcriptional regulation of SPMs remains largely unknown.

In addition to transcriptional regulation, signaling factors derived from the microenvironment also play an essential role in promoting the development and phenotype of tissue-resident macrophages. For example, TGF-β1 signaling is required for the development of the microglia population and to regulate a microglia expression program through the Smad tissue factors (78–80). Heme has been shown to induce Spi-c, a transcription factor important for red pulp macrophage development (81, 82). Finally, in PerC, omentum-derived RA promotes the expression of GATA-6 in the LPM subset, determining its localization and functions (40), even if the factors that maintain the SPM pool under steady state conditions still remain to be elucidated.

Dynamics and Function of Peritoneal Macrophage Subsets

Mouse PerC is a compartment where many cell types co-habitat and interact, similar to the secondary lymphoid organs. In addition, PerC is a unique body compartment that contains B-1 cells (83). Under steady state conditions, the peritoneal cells comprise LPMs, SPMs, B-1 cells, conventional B-2 cells, T cells, NK cells, DCs, and granulocytes (mostly eosinophils) (31, 35). B1 cells constitute the majority of the PerC cell population, whereas the SPM and LPM frequencies represent 30–35% of total peritoneal cells (31, 35) (**Figure 2A**). However, after inflammatory or infectious stimuli, there is a dramatic alteration in cell numbers and the frequencies of each of PerC cell subpopulation. With regard to the myeloid compartment, modifications in PerC cell composition include the disappearance of LPMs, increases in SPM frequency and numbers, and a massive recruitment of inflammatory monocytes (24, 31, 35, 36, 40) (**Figure 2B**).

The "macrophage disappearance reaction" (MDR) in PerC has been extensively described during delayed-type hypersensitivity (DTH) and acute inflammatory processes (84). MDR has been associated with cell death, emigration to draining lymph nodes, or adherence of macrophages to structural tissues. LPMs are the unique peritoneal macrophage subset that disappears from PerC, which is attributed not to cell death but rather to their migration to the omentum (31, 40). LPM disappearance in response to inflammatory stimuli is accompanied by an increase in SPM and inflammatory monocyte numbers (24, 31, 35, 36, 40) (**Figure 2B**), and has been correlated with the renewal and improvement of immune conditions of the PerC (35). Adherent peritoneal cells from naive mice, which are composed primarily of LPM, exhibit

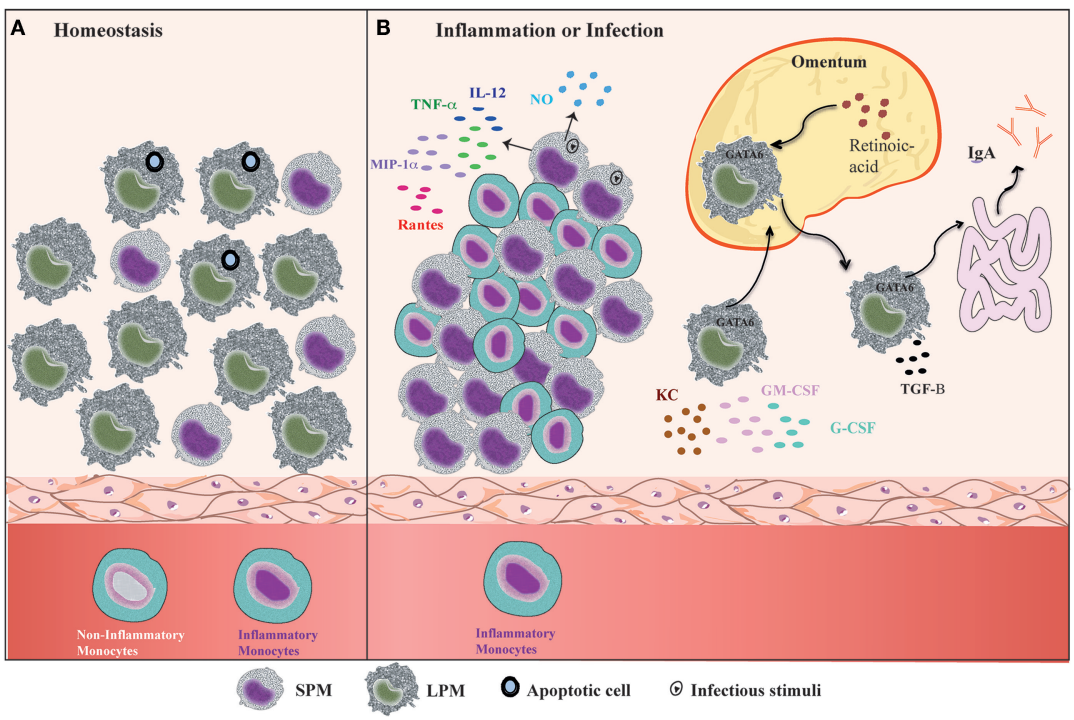

FIGURE 2 | Summary of the dynamic of peritoneal macrophage subsets. **(A)** Under homeostatic conditions, peritoneal macrophages comprise two subsets LPMs and SPMs (31). LPMs, which are the major peritoneal macrophage population, appear to be responsible for phagocytosis of apoptotic cell and tissue repair (36). **(B)** At the outset of inflammation, the myeloid compartment is modified in general by disappearance of LPMs, increase of SPMs numbers, and monocytes influx (31, 35, 36, 40). The changes in the myeloid cells from zymosan, *T. cruzi*, and LPS stimulated or thioglicollate-elicited PerC result in the gain of immune state (35, 36). SPMs from zymosan and *T. cruzi* stimulated mice contribute to effector function of PerC through secretion of high levels of NO and presence of IL-12-producing cells (35). In response to LPS *in vivo*, SPMs produce several inflammatory cytokines, such as IL-12, MIP-1α, TNF-α, and RANTES, whereas LPMs produce enhanced amounts of G-CSF, GM-CSF, and KC (36). LPMs, which migrate to omentum by a retinoic acid and GATA-6-dependent way in response to *in vivo* LPS stimulation or vitamin-A deprivation, return to PerC and appear to be correlated with GALT-independent and TGF-β2-dependent IgA production by B-1 cells in the intestine (40).

a high frequency of cells stained for β-galactosamine (β-gal), a senescence marker (85–87). These cells are unable to secrete NO in response to LPS challenge (35). In contrast, adherent peritoneal cells from *Trypanosoma cruzi* or zymosan-stimulated mice in which the main cell population constitutes SPMs and monocytes ($F4/80^{low}MHCII^{int}Ly\text{-}6C^{+}$), respectively, display a significant reduction in the frequency of β-gal-positive cells and secrete high levels of NO in response to LPS (35). The frequency of IL-12-producing cells after *in vitro* LPS plus IFN-γ stimulation was also higher within myelo-monocytic cells from mice exposed to zymosan and *T. cruzi* than the frequencies of IL-12-producing cells found in unstimulated mice (35). In response to *Staphylococcus epidermidis* cell-free (SES) supernatant *in vivo* stimulation, $F4/80^{low}CD11b^{+}$ cells (consisting of SPMs and DCs) produced enhanced levels of IL-1β, IL-1α, TNF-α, and IL-12 in the presence or absence of subsequent SES treatment (37). In contrast, the supernatants of adherent cells from naïve mice treated with SES were found to contain high levels of MCP-1, MCP-1α, MIP-1β, and G-CSF (37). It is important to note that 4 days after thioglycollate injection, peritoneal cells, an extensively studied cell population (88–91), also consist primarily of SPMs and inflammatory monocytes (31, 40). The increase in SPM numbers and the influx of inflammatory monocytes that will give rise to SPMs greatly contribute to the improvement of the capacity of PerC to deal with inflammatory stimuli. Indeed, although neither SPMs nor LPMs produce significant levels of pro- or anti-inflammatory cytokines under steady state conditions (35–37), SPMs appear to develop a pro-inflammatory profile in response to *in vitro* stimuli. SPMs produced high levels of TNF-α, MIP-1α, and RANTES in response to LPS, whereas LPMs were the unique population that produced abundant levels of G-CSF, GM-CSF, and KC in response to the same stimulus (36) (**Figure 2B**).

The NO secretion and pro-inflammatory cytokine production are the most important functions of activated macrophages by inflammatory stimulation and assigns the M1 profile (13, 34, 92–97). The functional profile of peritoneal macrophages was previous studied by our group and others (33, 34). Peritoneal macrophages from Th1-prone mouse strains (C57BL/6 and B10.A) are easily activated to produce NO in response to rIFN-γ or LPS, characterizing the M1 profile. In contrast, macrophages from Th2-prone mouse strains (BALB/c and DBA/2) exhibit a weak NO response as a consequence of high levels of spontaneously secreted TGF-β1 (34). Moreover, the cells from C57BL/6 IL-12p40-deficient mice have a bias toward the M2 profile, indicating that IL-12 is required for M1 polarization of peritoneal macrophages (33). Although LPMs from naïve mice can produce NO after *in vitro* LPS stimulation, SPMs produce higher levels of NO than LPMs following *in vivo* LPS stimulation. The NO secretion by LPMs was also detected by flow cytometry in *Escherichia coli* inoculated mice (31), whereas nitrite was not produced *in vitro* by LPS-stimulated adherent peritoneal cells from control mice, which is composed mainly by LPMs (35). In addition, adherent cells obtained 48 h after *T. cruzi* infection, which are mostly composed by SPMs, were the unique source of NO without *in vitro* subsequent challenge with LPS (35). In resume, the SPM and LPM subsets cannot be accommodated in the M1/M2 framework considering the NO secretion. However, considering phagocytic assays, SPMs appear to develop an efficient profile to control infections as M1 macrophages, whereas LPMs assume a role in the maintenance of PerC physiological conditions as M2 or alternative macrophages. Despite the preserved phagocytic ability of LPMs, higher numbers of zymosan and *E. coli* were found inside of SPMs at early time points after i.p. injection (31, 35). Conversely, at 1 h after challenge, LPMs appear to present a higher phagocytic index of apoptotic thymocytes in comparison to SPMs (36) (**Figure 2A**).

In addition, it was recently demonstrated that LPMs have a unique ability to induce gut-associated lymphoid tissue (GALT)-independent IgA production by peritoneal B-1 cells (40) (**Figure 2B**). RA and TGF-β2 are the most critical factors to induce IgA class switching, and the production of TGF-β2 is regulated by the *Tgfb2* and *Ltbp1* genes, which are expressed by LPMs in a GATA-6-dependent manner. This process is regulated by the abundant presence of RA in the omentum, which is responsible for the induction of GATA-6 expression in LPMs that migrates to this tissue. The dynamic of LPM migration between the PerC and the omentum after the stimulation of PerC is correlated with their disappearance and the return to basal numbers of LPMs later after stimulation with LPS, zymosan, and thioglycollate (24, 31, 35, 36, 39, 40). This observation suggests that LPMs can return to PerC to resolve an infectious or inflammatory process. Therefore, the presence of two specialized macrophage subsets in PerC is crucial to maintain the health of this compartment under different situations.

Concluding Remarks

Peritoneal macrophages represent one of the most studied macrophage populations. However, the existence of two phenotypically and functionally distinct subsets, LPMs and SPMs, residing in the PerC was recognized recently (31). In the last year, great advances in our understanding of the transcriptional regulation of peritoneal macrophages have brought novel insights into the identification of LPMs and SPMs (39, 40). GATA-6, an LPM-restricted transcription factor, regulates many PMSGs, including those related to the maintenance of LPMs in PerC (40) and those that determine their function (40), metabolism, proliferation, and cell survival (39, 77). Under steady state conditions, LPMs appear to originate independently from hematopoietic precursors and retained the ability to proliferate *in situ*, maintaining physiological numbers (26, 36). Conversely, SPMs appear to originate from circulating monocytes (31, 36, 40), and their numbers increase remarkably under inflammatory conditions. Of note, SPMs together with their precursor, the inflammatory monocyte population, are the major myeloid populations present in elicited PerC, and are an excellent resource to study the biology of inflammatory macrophages. SPMs and LPMs exhibit specialized functions in the PerC, where SPMs present a pro-inflammatory functional profile, and LPMs appear to have a role in the maintenance of PerC physiological conditions. Moreover, the particular interactions between macrophage subsets and other peritoneal cell populations appear to play crucial roles in PerC immune state. Although the consequences of the crosstalk between SPMs and peritoneal T and B lymphocytes remain to be clarified, LPMs are

required for GALT-independent and RA-dependent IgA production by peritoneal B-1 cells (40). Finally, the elucidation of the influence of soluble factors and the microbiota on the maintenance of LPM/SPM ratios in PerC, and the role of these subsets in the systemic immune response are the future challenges for this field.

References

1. Taylor PR, Gordon S. Monocyte heterogeneity and innate immunity. *Immunity* (2003) **19**(1):2–4. doi:10.1016/S1074-7613(03)00178-X
2. Gordon S. The macrophage: past, present and future. *Eur J Immunol* (2007) **37**(Suppl 1):S9–17. doi:10.1002/eji.200737638
3. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature* (2013) **496**(7446):445–55. doi:10.1038/nature12034
4. Metchnikoff E. *Leçons sur la Pathologie Comparée de l'inflammation faites à l'Institut Pasteur en 1891*. Paris: Masson (1892).
5. Van M. [Elie Metchnikoff, 1845-1916]. *Voeding* (1964) **25**:351–6.
6. Aschoff L. Das reticuloendotheliale system. *Ergeb Inn Med Kinderheilkd* (1924) **26**:1–117.
7. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ* (1972) **46**(6):845–52.
8. van Furth R. Current view of the mononuclear phagocyte system. *Haematol Blood Transfus* (1981) **27**:3–10.
9. van Furth R. The mononuclear phagocyte system. *Verh Dtsch Ges Pathol* (1980) **64**:1–11.
10. Hume DA, Ross IL, Himes SR, Sasmono RT, Wells CA, Ravasi T. The mononuclear phagocyte system revisited. *J Leukoc Biol* (2002) **72**(4):621–7.
11. Hume DA. The mononuclear phagocyte system. *Curr Opin Immunol* (2006) **18**(1):49–53. doi:10.1016/j.coi.2005.11.008
12. Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ. Unravelling mononuclear phagocyte heterogeneity. *Nat Rev Immunol* (2010) **10**(6):453–60. doi:10.1038/nri2784
13. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* (2005) **5**(12):953–64. doi:10.1038/nri1733
14. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**(8):571–8. doi:10.1038/nri3712
15. Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol* (2009) **27**:669–92. doi:10.1146/annurev.immunol.021908.132557
16. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science* (2010) **327**(5966):656–61. doi:10.1126/science.1178331
17. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol* (2013) **14**:986–95. doi:10.1038/ni.2705
18. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol* (2014) **14**(6):392–404. doi:10.1038/nri3671
19. Ginhoux F, Merad M. Ontogeny and homeostasis of Langerhans cells. *Immunol Cell Biol* (2010) **88**(4):387–92. doi:10.1038/icb.2010.38
20. van Furth R. [The origin of mononuclear phagocytes]. *Ned Tijdschr Geneeskd* (1967) **111**(48):2208.
21. van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. *J Exp Med* (1968) **128**(3):415–35. doi:10.1084/jem.128.3.415
22. van Furth R. Origin and kinetics of monocytes and macrophages. *Semin Hematol* (1970) **7**(2):125–41.
23. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) **330**(6005):841–5. doi:10.1126/science.1194637
24. Davies LC, Rosas M, Smith PJ, Fraser DJ, Jones SA, Taylor PR. A quantifiable proliferative burst of tissue macrophages restores homeostatic macrophage populations after acute inflammation. *Eur J Immunol* (2011) **41**(8):2155–64. doi:10.1002/eji.201141817
25. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**(6077):86–90. doi:10.1126/science.1219179
26. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**(1):79–91. doi:10.1016/j.immuni.2012.12.001
27. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**(4):792–804. doi:10.1016/j.immuni.2013.04.004
28. Auffray C, Fogg DK, Narni-Mancinelli E, Senechal B, Trouillet C, Saederup N, et al. CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. *J Exp Med* (2009) **206**(3):595–606. doi:10.1084/jem.20081385
29. Sieweke MH, Allen JE. Beyond stem cells: self-renewal of differentiated macrophages. *Science* (2013) **342**(6161):1242974. doi:10.1126/science.1242974
30. Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. *Annu Rev Immunol* (2005) **23**:901–44. doi:10.1146/annurev.immunol.23.021704.115816
31. Ghosn EE, Cassado AA, Govoni GR, Fukuhara T, Yang Y, Monack DM, et al. Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. *Proc Natl Acad Sci U S A* (2010) **107**(6):2568–73. doi:10.1073/pnas.0915000107
32. Schleicher U, Hesse A, Bogdan C. Minute numbers of contaminant CD8+ T cells or CD11b+CD11c+ NK cells are the source of IFN-gamma in IL-12/IL-18-stimulated mouse macrophage populations. *Blood* (2005) **105**(3):1319–28. doi:10.1182/blood-2004-05-1749
33. Bastos KR, Alvarez JM, Marinho CR, Rizzo LV, Lima MR. Macrophages from IL-12p40-deficient mice have a bias toward the M2 activation profile. *J Leukoc Biol* (2002) **71**(2):271–8.
34. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* (2000) **164**(12):6166–73. doi:10.4049/jimmunol.164.12.6166
35. Cassado Ados A, de Albuquerque JA, Sardinha LR, Buzzo Cde L, Faustino L, Nascimento R, et al. Cellular renewal and improvement of local cell effector activity in peritoneal cavity in response to infectious stimuli. *PLoS One* (2011) **6**(7):e22141. doi:10.1371/journal.pone.0022141
36. Cain DW, O'Koren EG, Kan MJ, Womble M, Sempowski GD, Hopper K, et al. Identification of a tissue-specific, C/EBPbeta-dependent pathway of differentiation for murine peritoneal macrophages. *J Immunol* (2013) **191**(9):4665–75. doi:10.4049/jimmunol.1300581
37. Dioszeghy V, Rosas M, Maskrey BH, Colmont C, Topley N, Chaitidis P, et al. 12/15-Lipoxygenase regulates the inflammatory response to bacterial products in vivo. *J Immunol* (2008) **181**(9):6514–24. doi:10.4049/jimmunol.181.9.6514
38. Rosas M, Thomas B, Stacey M, Gordon S, Taylor PR. The myeloid 7/4-antigen defines recently generated inflammatory macrophages and is synonymous with Ly-6B. *J Leukoc Biol* (2010) **88**(1):169–80. doi:10.1189/jlb.0809548
39. Rosas M, Davies LC, Giles PJ, Liao CT, Kharfan B, Stone TC, et al. The transcription factor Gata6 links tissue macrophage phenotype and proliferative renewal. *Science* (2014) **344**(6184):645–8. doi:10.1126/science.1251414
40. Okabe Y, Medzhitov R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell* (2014) **157**(4):832–44. doi:10.1016/j.cell.2014.04.016
41. Wang C, Yu X, Cao Q, Wang Y, Zheng G, Tan TK, et al. Characterization of murine macrophages from bone marrow, spleen and peritoneum. *BMC Immunol* (2013) **14**:6. doi:10.1186/1471-2172-14-6
42. Davies LC, Rosas M, Jenkins SJ, Liao CT, Scurr MJ, Brombacher F, et al. Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. *Nat Commun* (2013) **4**:1886. doi:10.1038/ncomms2877

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43. Dahdah A, Gautier G, Attout T, Fiore F, Lebourdais E, Msallam R, et al. Mast cells aggravate sepsis by inhibiting peritoneal macrophage phagocytosis. *J Clin Invest* (2014) **124**(10):4577–89. doi:10.1172/JCI75212
44. Machado MCC, Coelho AMM. *Role of Peritoneal Macrophages on Local And Systemic Inflammatory Response in Acute Pancreatitis*. São Paulo: InTech (2012). doi:10.5772/25639
45. Cohn ZA. Determinants of infection in the peritoneal cavity. I. Response to and fate of *Staphylococcus aureus* and *Staphylococcus albus* in the mouse. *Yale J Biol Med* (1962) **35**:12–28.
46. Cohn ZA. Determinants of infection in the peritoneal cavity. II. Factors influencing the fate of *Staphylococcus aureus* in the mouse. *Yale J Biol Med* (1962) **35**:29–47.
47. Cohn ZA. Determinants of infection in the peritoneal cavity. III. The action of selected inhibitors on the fate of *Staphylococcus aureus* in the mouse. *Yale J Biol Med* (1962) **35**:48–61.
48. Steinman RM, Moberg CL. Zanvil Alexander Cohn 1926-1993. *J Exp Med* (1994) **179**(1):1–30. doi:10.1084/jem.179.1.1
49. Taylor PR, Brown GD, Geldhof AB, Martinez-Pomares L, Gordon S. Pattern recognition receptors and differentiation antigens define murine myeloid cell heterogeneity ex vivo. *Eur J Immunol* (2003) **33**(8):2090–7. doi:10.1002/eji.200324003
50. Hickstein DD, Ozols J, Williams SA, Baenziger JU, Locksley RM, Roth GJ. Isolation and characterization of the receptor on human neutrophils that mediates cellular adherence. *J Biol Chem* (1987) **262**(12):5576–80.
51. Petty HR, Todd RF III. Receptor-receptor interactions of complement receptor type 3 in neutrophil membranes. *J Leukoc Biol* (1993) **54**(5):492–4.
52. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. *Nat Rev Immunol* (2002) **2**(3):151–61. doi:10.1038/nri746
53. Kantor AB, Stall AM, Adams S, Herzenberg LA. Differential development of progenitor activity for three B-cell lineages. *Proc Natl Acad Sci U S A* (1992) **89**(8):3320–4. doi:10.1073/pnas.89.8.3320
54. Ghosn EE, Yang Y, Tung J, Herzenberg LA. CD11b expression distinguishes sequential stages of peritoneal B-1 development. *Proc Natl Acad Sci U S A* (2008) **105**(13):5195–200. doi:10.1073/pnas.0712350105
55. Hume DA, Gordon S. Mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80. Identification of resident macrophages in renal medullary and cortical interstitium and the juxtaglomerular complex. *J Exp Med* (1983) **157**(5):1704–9. doi:10.1084/jem.157.5.1704
56. Hume DA, Loutit JF, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: macrophages of bone and associated connective tissue. *J Cell Sci* (1984) **66**:189–94.
57. Hume DA, Perry VH, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80: macrophages associated with epithelia. *Anat Rec* (1984) **210**(3):503–12. doi:10.1002/ar.1092100311
58. Bedoret D, Wallemacq H, Marichal T, Desmet C, Quesada Calvo F, Henry E, et al. Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice. *J Clin Invest* (2009) **119**(12):3723–38. doi:10.1172/JCI39717
59. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* (2013) **210**(10):1977–92. doi:10.1084/jem.20131199
60. Hume DA, Robinson AP, MacPherson GG, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80. Relationship between macrophages, Langerhans cells, reticular cells, and dendritic cells in lymphoid and hematopoietic organs. *J Exp Med* (1983) **158**(5):1522–36. doi:10.1084/jem.158.5.1522
61. Hume DA, Perry VH, Gordon S. Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. *J Cell Biol* (1983) **97**(1):253–7. doi:10.1083/jcb.97.1.253
62. Hume DA, Halpin D, Charlton H, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: macrophages of endocrine organs. *Proc Natl Acad Sci U S A* (1984) **81**(13):4174–7. doi:10.1073/pnas.81.13.4174
63. Austyn JM, Gordon S. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur J Immunol* (1981) **11**(10):805–15. doi:10.1002/eji.1830111013
64. Ghosn EE, Yang Y, Tung J, Herzenberg LA, Herzenberg LA. CD11b expression distinguishes sequential stages of peritoneal B-1 development. *Proc Natl Acad Sci U S A* (2008) **105**(13):5195–200. doi:10.1073/pnas.0712350105
65. Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, Littman DR, et al. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* (2006) **311**(5757):83–7. doi:10.1126/science.1117729
66. Landsman L, Varol C, Jung S. Distinct differentiation potential of blood monocyte subsets in the lung. *J Immunol* (2007) **178**(4):2000–7. doi:10.4049/jimmunol.178.4.2000
67. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* (2003) **19**(1):71–82. doi:10.1016/S1074-7613(03)00174-2
68. Chorro L, Geissmann F. Development and homeostasis of 'resident' myeloid cells: the case of the Langerhans cell. *Trends Immunol* (2010) **31**(12):438–45. doi:10.1016/j.it.2010.09.003
69. Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, et al. C-myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* (2015) **42**(4):665–78. doi:10.1016/j.immuni.2015.03.011
70. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* (2007) **10**(12):1538–43. doi:10.1038/nn2014
71. Chorro L, Sarde A, Li M, Woollard KJ, Chambon P, Malissen B, et al. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. *J Exp Med* (2009) **206**(13):3089–100. doi:10.1084/jem.20091586
72. Zigmond E, Jung S. Intestinal macrophages: well educated exceptions from the rule. *Trends Immunol* (2013) **34**(4):162–8. doi:10.1016/j.it.2013.02.001
73. Parwaresch MR, Wacker HH. Origin and kinetics of resident tissue macrophages. Parabiosis studies with radiolabelled leucocytes. *Cell Tissue Kinet* (1984) **17**(1):25–39.
74. Melnicoff MJ, Horan PK, Breslin EW, Morahan PS. Maintenance of peritoneal macrophages in the steady state. *J Leukoc Biol* (1988) **44**(5):367–75.
75. Daems WT, de Bakker JM. Do resident macrophages proliferate? *Immunobiology* (1982) **161**(3–4):204–11. doi:10.1016/S0171-2985(82)80075-2
76. Wijffels JF, Hendrickx RJ, Steenbergen JJ, Eestermans IL, Beelen RH. Milky spots in the mouse omentum may play an important role in the origin of peritoneal macrophages. *Res Immunol* (1992) **143**(4):401–9. doi:10.1016/S0923-2494(05)80072-0
77. Gautier EL, Ivanov S, Williams JW, Huang SC, Marcelin G, Fairfax K, et al. Gata6 regulates aspartoacylase expression in resident peritoneal macrophages and controls their survival. *J Exp Med* (2014) **211**(8):1525–31. doi:10.1084/jem.20140570
78. Makwana M, Jones LL, Cuthill D, Heuer H, Bohatschek M, Hristova M, et al. Endogenous transforming growth factor beta 1 suppresses inflammation and promotes survival in adult CNS. *J Neurosci* (2007) **27**(42):11201–13. doi:10.1523/JNEUROSCI.2255-07.2007
79. Abutbul S, Shapiro J, Szaingurten-Solodkin I, Levy N, Carmy Y, Baron R, et al. TGF-beta signaling through SMAD2/3 induces the quiescent microglial phenotype within the CNS environment. *Glia* (2012) **60**(7):1160–71. doi:10.1002/glia.22343
80. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. *Nat Neurosci* (2014) **17**(1):131–43. doi:10.1038/nn.3599
81. Kohyama M, Ise W, Edelson BT, Wilker PR, Hildner K, Mejia C, et al. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. *Nature* (2009) **457**(7227):318–21. doi:10.1038/nature07472
82. Haldar M, Kohyama M, So AY, Kc W, Wu X, Briseno CG, et al. Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. *Cell* (2014) **156**(6):1223–34. doi:10.1016/j.cell.2014.01.069
83. Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nat Rev Immunol* (2011) **11**(1):34–46. doi:10.1038/nri2901
84. Barth MW, Hendrzak JA, Melnicoff MJ, Morahan PS. Review of the macrophage disappearance reaction. *J Leukoc Biol* (1995) **57**(3):361–7.

85. Lloberas J, Celada A. Effect of aging on macrophage function. *Exp Gerontol* (2002) **37**(12):1325–31. doi:10.1016/S0531-5565(02)00125-0
86. Herrero C, Sebastian C, Marques L, Comalada M, Xaus J, Valledor AF, et al. Immunosenescence of macrophages: reduced MHC class II gene expression. *Exp Gerontol* (2002) **37**(2–3):389–94. doi:10.1016/S0531-5565(01)00205-4
87. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* (1995) **92**(20):9363–7. doi:10.1073/pnas.92.20.9363
88. Takahashi M, Galligan C, Tessarollo L, Yoshimura T. Monocyte chemoattractant protein-1 (MCP-1), not MCP-3, is the primary chemokine required for monocyte recruitment in mouse peritonitis induced with thioglycollate or zymosan A. *J Immunol* (2009) **183**(5):3463–71. doi:10.4049/jimmunol.0802812
89. Cohn ZA. Activation of mononuclear phagocytes: fact, fancy, and future. *J Immunol* (1978) **121**(3):813–6.
90. Leijh PC, van Zwet TL, ter Kuile MN, van Furth R. Effect of thioglycolate on phagocytic and microbicidal activities of peritoneal macrophages. *Infect Immun* (1984) **46**(2):448–52.
91. Lagasse E, Weissman IL. Flow cytometric identification of murine neutrophils and monocytes. *J Immunol Methods* (1996) **197**(1–2):139–50. doi:10.1016/0022-1759(96)00138-X
92. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* (2002) **23**(11):549–55. doi:10.1016/S1471-4906(02)02302-5
93. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* (2003) **3**(1):23–35. doi:10.1038/nri978
94. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* (2004) **25**(12):677–86. doi:10.1016/j.it.2004.09.015
95. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol* (2009) **27**:451–83. doi:10.1146/annurev.immunol.021908.132532
96. Mosser DM. The many faces of macrophage activation. *J Leukoc Biol* (2003) **73**(2):209–12. doi:10.1189/jlb.0602325
97. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* (2008) **8**(12):958–69. doi:10.1038/nri2448

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The complex myeloid network of the liver with diverse functional capacity at steady state and in inflammation

Christoph Eckert, Niklas Klein, Miroslaw Kornek and Veronika Lukacs-Kornek*

Department of Medicine II, Saarland University Medical Center, Homburg, Germany

Edited by:
Florent Ginhoux, Singapore Immunology Network, Singapore

Reviewed by:
Frank Tacke, University Hospital Aachen, Germany
Charles-Antoine Dutertre, Duke-NUS Graduate Medical School, Singapore

****Correspondence:***
Veronika Lukacs-Kornek, Department of Medicine II, Saarland University Medical Center, Kirrbergerstr. 100, Homburg 66424, Germany
e-mail: veronika.lukacs-kornek@uniklinikum-saarland.de

In recent years, it has been an explosion of information regarding the role of various myeloid cells in liver pathology. Macrophages and dendritic cell (DC) play crucial roles in multiple chronic liver diseases such as fibrosis and non-alcoholic fatty liver disease (NAFLD). The complexity of myeloid cell populations and the missing exclusive marker combination make the interpretation of the data often extremely difficult. The current review aims to summarize the multiple roles of macrophages and DCs in chronic liver diseases, especially pointing out how these cells influence liver immune and parenchymal cells thereby altering liver function and pathology. Moreover, the review outlines the currently known marker combinations for the identification of these cell populations for the study of their role in liver immunology.

Keywords: dendritic cells, Kupffer cells, liver fibrosis, NASH, inflammatory monocytes

LIVER AS AN IMMUNE MILIEU

The liver functions as a metabolic center to ensure the proper processing of nutrients and the clearance of toxins; yet, plays multiple roles in systemic immune responses and in immune surveillance. The liver receives blood from both the systemic circulation and the intestine that mixes within the liver sinusoids (1). Approximately, two-third of the hepatic blood flow procures from the oxygen rich arteria hepatica and one-third is from the vena porta carrying microbial and food-derived antigens and molecules (1, 2). The mixed blood travels through the sinusoids that are specialized blood vessels lined by the liver sinusoidal endothelial cells (LSECs). LSECs assemble a discontinuous endothelium that is in contact with various passenger and organ-resident immune cells (3). Besides LSECs, the liver contains other parenchymal cells such as hepatocytes and hepatic stellate cells (HSCs). The activation status and extracellular matrix production of HSCs are critical for the progression of multiple liver diseases (4, 5). Importantly, these liver parenchymal cells interact with the variety of immune cells, influence memory T cells, respond to danger signals, and additionally take on the role of antigen presenting cells (APCs) within the liver (6, 7). As APCs, they present antigens in the context of immunosuppressive cytokines and inhibitory surface molecules resulting largely in tolerance (6, 7). The liver also encompasses large populations of hematopoetic cells such as innate lymphocytes (NK, NKT cells, and γδT cells) and myeloid cells [dendritic cells (DCs) and macrophages] (6). Multiple cross-talks exist between hematopoetic cells and liver parenchymal cells at steady state and during injury. This review focuses on the physiological and pathological roles of liver DCs and macrophages paying special attention to chronic liver diseases such as fibrosis and non-alcoholic fatty liver disease (NAFLD).

Abbreviations: APC, antigen presenting cell; BDL, bile duct ligation; BM, bone marrow; DCs, dendritic cells; Batf3, basic leucine zipper transcription factor, ATF-like 3; CCL_4, carbon tetrachloride; CCR9, chemokine (C–C motif) receptor 9; CTL, cytotoxic T lymphocyte; DNGR-1, DC NK lectin group receptor-1; DT, diphtheria toxin; DTR, diphtheria toxin receptor; ECM, extracellular matrix; FA, fatty acid; Flt3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; HSC, hepatic stellate cells; HL-DC, high-lipid liver dendritic cell; Id2, inhibitor of DNA binding 2; IL, interleukin; KC, Kupffer cells; LL-DC, low-lipid liver dendritic cell; LSEC, liver sinusoidal endothelial cells; LPS, lipopolysaccharide; MCD, methionine choline deficient; MCP-1, monocyte chemotactic protein-1; MMP-9, matrix metalloproteinase-9; MMP13, matrix metalloproteinase-13; NASH, non-alcoholic steatohepatitis; pDC, plasmacytoid dendritic cell; PDL-1, programed cell death 1 ligand; TNFα, tumor necrosis factor-alpha; TAA, thioacetamide; TLR, toll like receptor; zbtb46, zinc finger and BTB domain containing.

LIVER DENDRITIC CELLS

Dendritic cells are present in all tissues and represent the major APCS within the body (8). They constantly sense their environment and capable of recognizing pathogens and various danger signals. Activation of DCs results in their maturation toward several functionally distinct "effector DCs" (9) that drive T cell responses, such as T helper cell differentiation, induction of CTL, and T cell tolerance (9). Additionally, DCs communicate with innate lymphocytes (e.g., NK, NKT cells), therefore, can influence both innate and adaptive immune responses (8).

Murine liver DC population, similarly as in most non-lymphoid organs (except the lamina propria and dermis), consists of three types of DCs (**Table 1**): the cDC1s (classical type 1 DCs), the cDC2s (classical type 2 DCs), and pDCs (10, 11). Despite of this categorization, in most liver studies, DCs are evaluated as either $CD11c^+$ or $MHCII^+$ cells. Although neither of the molecules pinpoint exclusively DCs, using these markers liver DCs are primarily located within the portal area and rarely scattered within the parenchyma (6). The cDC1 cells resemble lymphoid tissue $CD8^+$ DCs, show migratory capacity in various non-lymphoid organs, and can efficiently cross-present cell-associated antigens (10, 11). Although the role of DC migration in liver pathology has not been explored in details, antigen injected or targeted to the

Table 1 | Summary of DC and macrophage population in healthy and injured liver.

Cell types	Murine	Human	Reference
Dendritic cells			
Classical Type 1 DCs (cDC1)	$CD45^+$ $PDCA1^-$ $CD11c^+$ $CD11b^-$ $CD103^+$ $MHCII^+$ $Langerin^{+/-}$ $F4/80^-$ $CX3CR1^-$	$CD45^+$ $HLA\text{-}DR^+$ $CD141^+$ $CD123^-$ $CD11c^+$ $CD14^-$	(8, 15)
Classical Type 2 DCs (cDC2)	$CD45^+$ $PDCA1^-$ $CD11c^+$ $CD11b^+$ $CD103^-$ $MHCII^+$ $F4/80^{+/-}$ $Langerin^-$ $CX3CR1^+$	$CD45^+$ $HLA\text{-}DR^+$ $CD1c^+$ $CD123^-$ $CD11c^+$ $CD141^-$ $CD14^+$	(10, 15–17)
pDCs	$CD45^+$ $PDCA1^+$ $CD11c^+$	$HLA\text{-}DR^+$ $CD123^+$ $CD11c^-$ $CD303^+$ $CD304^+$	(17)
pre-DCs	$CD45^+$ $CD11c^+$ $MHCII^-$ $Flt3^{+/-}$	ND	(18)
Macrophages			
KCs	$CD68^+$ $F4/80^+$ $CD11b^{low}$ $Ly6C^{low}$ $Ly6G^-$ $TLR4^+$ $TLR9^+$	$CD68^+$	(19–22)
$Ly6C^{hi}$ classical monocytes	$F4/80^+$ $CD11b^{hi}$ $Ly6C^{hi}$ $Gr1^+$ $CX3CR1^+$ $CCR2^+$	$CD14^{hi}$ $CD16^-$	(23, 24)
M1 inflammatory macrophages/monocytes	$F4/80^+$ $CCR9^+$ iNOS+ galectin-3^+	$CD14^+$ $CD16^+$(it is not yet clarified how they differ from non-classical monocytes)	(25–27)
Restorative macrophages	$F4/80^+$ $CD11b^{low}$ $Ly6C^{low}$	$CD14^+$ $CD16^+$	(24, 26, 28)

DCs and macrophages are classified according to the recently suggested nomenclature based on the ontogeny of these cells (15). The M1 type monocyte/macrophage population present in liver injury and the restorative macrophages during resolution could not be incorporated in this nomenclature as their origin and relation to monocytes and resident liver macrophages (KCs) need further clarification in the future. DCs, dendritic cells; KCs, Kupffer cells; pDCs, plasmacytoid DCs; pre-DCs, precursor DCs.

liver reaches the draining LN and induces T cell activation (12, 13). Additionally, migratory DCs could be identified within the portal lymphatic vessels in electronmicroscopy analyses (1, 14).

The cDC2s within non-lymphoid organs are heterogeneous and partially monocyte lineage derived (10, 11). Their specific role is less understood in non-lymphoid organs, involving the liver as well. While the development of this subset depends in most non-lymphoid organs on the presence of FLT3L and M-CSF, in the liver, these cells are not exclusively dependent on these growth factors but yet on an unidentified molecule (10). The liver, similarly to other non-lymphoid tissues, contains not only fully differentiated DCs but precursor DC population as well. From these pre-DCs, either FLT3L or GM-CSF can induce liver DC development bestowing DC homeostasis *in situ* (29, 30).

Functionally, $CD11c^+$ cells isolated from healthy mouse liver are less mature, have lower capacity to endocytose antigen, and induce less efficient allogenic T cell activation as secondary lymphoid organ (SLO)-derived DCs (31, 32). The inhibitory/tolerogenic capacity of liver DCs could be attributed to the specific microenvironment provided by parenchymal cells of the liver. Fibroblastic and $VCAM^+$ cells derived from the liver could induce hematopoetic progenitor cells to differentiate toward tolerogenic DCs *in vitro* that can inhibit experimental autoimmune hepatitis (33). It is assumed that circulatory DCs during their translocation within the liver sinusoids toward the lymphatics receive such tolerogenic education from liver parenchymal cells (14, 34). Yet, its *in vivo* relevance needs to be elucidated.

Freshly isolated murine liver $CD11c^+$ cells promote Th2 rather than Th1 T cell differentiation and via interacting with NK cells induce regulatory T cell (Treg) development (35, 36). Moreover, liver DCs produce increased amount of IL-10, IL-27 but less IL-12 upon LPS stimuli (37, 38). This hyporesponsive behavior toward TLR stimuli, known as endotoxin tolerance, involves LPS/TLR4 but also extends toward other TLRs (6). This is especially important, as the liver is constantly exposed to gut derived microbial products. The breakdown in this tolerance could be observed in colitis where pro-inflammatory DC/macrophage population expands within the liver due to the increased amount of bacterial products present in the portal blood. This creates an inflammatory environment in the liver despite the absence of direct liver damage (39). The tolerant state toward TLRs is an active process and involves the action of various negative regulators of the TLR signaling pathway (6). Interestingly, under steady state, liver DCs rather respond to ECM stimuli (collagen-type I, laminin, fibronectin) that induces MHC-II upregulation and maturation of GM-CSF expanded liver DCs *in vitro* (40).

In humans, the cDC2 cells ($CD11c^+$ $BDCA1^+$) are the most abundant in the liver and they exhibit similar immature, tolerogenic capacity as their murine counterpart (16, 41) (**Table 1**). The cDC1 cell population that expresses $CD141^+$ has been recently identified as a counterpart of murine $CD8\alpha^+$ cells (42). These cells induce pro-inflammatory allogeneic MLRs, resulting in IFN-γ and IL-17 production by activated T cells (17). Importantly, as opposite to cDC2s and pDCs, cDC1s (identified in the study as $CD141^+$ cells) were markedly decreased during liver diseases but among the DC-subsets produced the highest level of IFN-λ (17). It is possible that functional differences are reflected among the DC subsets and each subset represents different aspects of liver immunity and tolerance. In line with this, a classification of murine liver DCs according to their lipid content distinguishes between immunogenic and tolerogenic liver DCs. Due to their acetyl-CoA carboxylase activity, HL-DCs (high lipid DCs) mount strong immunogenic CTLs while the LL-DCs (low lipid DCs) with low lipid content are tolerogenic (43). Notably, the marker combinations used for this study showed that both HL-DCs and LL-DCs include multiple DC-subsets distinguished by currently known

surface markers and were not restricted to one specific subset. Novel surface molecules are needed to specifically explore their functional diversity.

pDCs are the major source of type-I IFN, regulate NK cell activity, and play important role in the induction of antiviral immunity (44, 45). The murine liver is especially rich in pDCs; yet, the human counterpart contains a smaller proportion of this population among all DCs (17) (**Table 1**). Under steady state condition, pDCs express low level of costimulatory molecules, are weak T cell stimulators, and induce apoptosis in activated T cells in a Treg dependent manner (46). Later could indicate a cellular interplay between pDCs and Tregs in the liver microenvironment in order to maintain the tolerogenic milieu. Accordingly, pDCs can induce efficient CD4 and CD8 T cell tolerance to orally administered antigens (47).

Microbial products, such as muramyl dipeptide present in the portal blood, upregulate PDL-1 in pDCs and reduce their response to TLR9 stimuli (48). This is another example for the TLR-mediated hyporesponsiveness ("endotoxin tolerance") in the liver. Strikingly, upon FLT3L treatment, the expanded liver pDCs display strong immunostimulatory properties (49). It is unclear whether this could be due to the expansion of a specific subpopulation of pDCs, or their modified interaction with Tregs, or the result of the complete rearrangement in the myeloid cell compartment, and the consequent imbalance in the tolerogenic milieu.

Taken together, the multiple DC subsets within the liver participate in guarding the tolerogenic environment and primarily skewed toward suppressing T cell responses and toward induction of Tregs. While DCs are the main APCs and inducers of T cell immunity in SLOs, within the liver environment the question still remains: how immunity can be induced by DCs in such suppressive microenvironment? Induction of immunity might be attributed to special DC subpopultaions such as the $CD141^+$ cDC1s in humans (17) and the $CD103^+$ cDC1s in mice (50). Moreover, the appearance of novel DC population, such as monocyte-derived DCs present in iMATEs (intrahepatic myeloid-cell aggregates for T cell population expansion), participates in efficient CLT expansion within the liver (51). Alternatively, immunity is induced by migratory DCs reaching the draining LN, thus, outside of the liver suppressive environment. In line with this, antigen specifically expressed in draining LN results in hepatitis inducing CD8 T cell activation, while the same antigen within the liver induces tolerance (52). Additionally, the liver can provide newly formed structures for T cell activation resulting in immunity. Portal tract associated lymphatic structures (PALPs) during *Propionibacterium acnes* granuloma formation and tertiary lymphoid structures in biliary cirrhosis represent locations where possible T and B cell activation takes place, respectively (53, 54).

KUPFFER CELLS – RESIDENT MACROPHAGE POPULATION OF THE LIVER

Kupffer cells (KCs) are tissue resident macrophages and they represent the largest hematopoetic cell population within the liver. They arise from yolk sac during fetal development (55), adjust themselves to the local microenvironment (56, 57), and renew their population at steady state locally throughout adult life with no or minimal contribution of hematopoetic progenitors or blood monocytes (58–60). In mice, KCs can be distinguished from monocytes among the $F4/80^+$ cells as $Ly6C^{low}$ $CD11b^{low}$ cell population (20, 21) (**Table 1**) and possess functional specifications according to their positioning within the sinusoid (61). Recent study could distinguish two KC functional groups: the one with higher phagocytosis capacity and the one with preference toward cytokine production (61, 62). Additionally, macrophages are functionally grouped into two classes M1 and M2. While such plain classification is questionable and often overstated, still provide a simple but distinguishable concept for functional categorization of these cells. M1 (termed classically activated) macrophages are pro-inflammatory, while the M2 (termed alternatively activated) macrophages are suppressive and involved in cellular repair (63). According to this, KCs belong to the M2 type of cells and play fundamental role in homeostasis, immune surveillance, and tissue repair (63).

Their importance as tolerogenic APCs in the liver microenvironment is demonstrated in liver transplantation where they prolong allograft survival (31). At steady state, they inhibit DC mediated T cell activation within the sinusoids and presentation of high affinity peptide by KCs results in deletional CD8 T cell tolerance (6, 64). Furthermore, they promote the suppressive capacity of Tregs toward hepatic antigens (65, 66).

As all tissue resident macrophages, KCs express a wide repertoire of receptors for the recognition of pathogens and danger signals such as Toll-like receptors, members of the inflammasome, and scavenger receptors (31). In the presence of TLR ligands such as LPS and CpG, KCs become immunogenic, and can induce CD8 T cell activation, and the generation of efficient CLT response (67, 68). Thus, during liver infection, they support the development of antimicrobial T cell responses. Unfortunately, KCs induce efficient CTL against antigens from the systemic circulation such as the case in influenza infection (69). This CTL response results in bystander hepatitis, often accompanying systemic viral infections. Besides CD8 T cell responses, recent study describes naive CD4 T cell activation in the murine liver by antigens expressed in hepatocytes. This process is independent from lymphoid tissue but dependent on clodronate-sensitive liver APC population possibly involving KCs as well (70). Thus, KCs participate in the generation of both CD4 and CD8 T cell responses.

Using their scavenger receptor repertoire, KCs are involved in the clearance of apoptotic cell debris and central to iron homeostasis (71). KCs interact with multiple immune cells within the sinusoids, such as Tregs, DCs, DC precursors, and innate lymphocytes (7, 53, 72, 73). After recognizing any danger signals, KCs primarily drive the influx of inflammatory leukocytes such as neutrophils and monocytes (63). Thus, KCs function as sentinels and central orchestrators of cellular processes in healthy and injured liver. Additionally, while they support the tolerogenic milieu within the liver, their presence also ensures the protection of the liver during pathogen invasion.

TOOLS TO STUDY LIVER DCs AND MACROPHAGES

In order to characterize the specific physiological and pathological roles of DCs and macrophages, various animal models, tools have been developed (**Table 2**) Among these models, there are mouse lines deficient in transcription factors that are responsible

Table 2 | Summary of the available models to study liver macrophages and DCs.

Animal model	Cell types affected	Liver fibrosis/NASH studies
Transcription factors		
Cfsr1$^{op/op}$, Cfsr1$^{-/-}$, Csfr2$^{-/-}$	Macrophages, monocytes, some DCs, granulocytes	ND
Batf3$^{-/-}$, ID2$^{-/-}$, IRF8$^{-/-}$	CD8^{+} DCs, CD103^{+} DC	ND
Flt3L$^{-/-}$, injection of FLT3L	CD8^{+} CD11b^{-}, CD11b^{+} DCs, pDCs	(75)
IRF2$^{-/-}$, IRF4$^{-/-}$	CD8^{-} CD11b^{+} DCs	ND
DTR system		
CD11c-DTR-short promoter-long promoter	DCs, plasmablast, some activate CD8 T cells, marginal zone macrophages, alveolar macrophages, some B cells All above + some NK and NKT, pDCs, monocyte-derived DCs	(75–78)
CD11b-DTR	Neutrophils, monocytes, eosinophils, macrophages, some DCs	(28, 79, 80)
CD169-DTR	Splenic MM macrophages, LN macrophages, BM macrophages, KC	ND
Langerin-DTR	Langerin^{+} dermal DCs, langerhans cells, some CD8^{+}DCs, and some CD103^{+} DCs	ND
Zbtb46-DTR	DCs and DC committed progenitors, monocytes (IL-4 and GM-CSF)	ND
Clodronate liposome mediated cell depletion	Macrophages, some DCs, monocytes	(81–86)
Reporter/Cre mouse lines		
CX3CR1-GFP	Macrophages, monocytes, some DCs	(87, 88)
Cfsr1-GFP (MacGreen)	Macrophages, monocytes, some DCs	
Lyz2-GFP/Lyz2-Cre	Macrophages, granulocytes	
Cfsr1-GFP	Macrophages, monocytes, some DCs	
CCR2-RFP	Monocytes, macrophages, memory T cells	
MHCII-EGFP	Macrophages, DC, B cells	
CD11c-YFP/CD11c Cre	See above	
Langerin-GFP	See above	
DNGR-1-GFP	DCs, pre-DCs	

ND, non-determined; DCs, dendritic cells; MM, marginal zone.

for the development of one or multiple subsets of myeloid cells. Due to the multiple cell types affected in these models, the broader impact of each of these genes makes it difficult to unequivocally pinpoint subset specific functions. Nevertheless, these transgenic animals helped significantly to establish broader understanding of macrophage and DC development and their role under steady state and inflammation (63, 74). However, just few of these models have been evaluated so far in fibrosis and non-alcoholic steatohepatitis (NASH) models (**Table 2**) but also in liver immunology. This might extend in the future as genetic model lacking cDC1s have just recently demonstrated that cDC1s respond to hepatotropic viral infection and are keys in the induction of anti-viral CD8 T cell response *in situ* (50).

The most frequently used cell-depleting tools in liver immunology are the clodronate liposome mediated depletion of mononuclear cells and the CD11c-/CD11b-DTR (diphtheria toxin receptor) transgenic system (87, 89). Clodronate-encapsulated liposomes are taken up by mononuclear cells and the clodronate bisphosphonate within the cell induces apoptosis that results in depletion of the phagocytic cell population. Multiple phagocytic cell types are affected using this depletion method such as KCs, macrophages, and some members of the DC population as well. Since more than one cell types are affected, the effects can be extrapolated to a group of cells and not to individual subtypes (89). Additionally, the release of inflammatory mediators (such as TNF) has been associated with this type of cell depletion further complicating the interpretation of experimental results (90).

The other widely used tool for liver biology is the CD11c-DTR-based depletion system. Here, the human diphtheria toxin receptor is expressed under the CD11c promoter and administration of diphtheria toxin results in the depletion of CD11c^{+} cells. This model is used to dissect the role of conventional DCs. The major disadvantage in this system is that multiple cell types are affected such as marginal zone macrophages, monocytes, activated CD8 T cells, NK cells, and plasmacytoid DCs (89). Two different CD11c-DTR mouse lines have been generated: the one encompassing only a short piece of the CD11c promoter (3) and the one with the full-length promoter (91). Although, they differ in the list of affected cell types, they gave important insights in the role of CD11c^{+} cells in liver immunology (**Table 2**). Novel DTR tools have been developed in recent years that aim to restrict the expression of DTR more specifically to DCs. The zbtb-46-DTR model uses the transcription factor zbtb46 that is exclusively expressed by DCs and DC-committed precursors (92). Unfortunately, zbtb46 is upregulated in monocytes stimulated with GM-CSF and IL-4, suggesting some limitations to

this promoter (93, 94). Another promising promoter is the DC NK lectin group receptor-1 (DNGR-1) that seems to be highly restricted to the DC linage (95). Not only for DCs but also for the study of macrophages, the perfect targeting tool still needs to be developed. The primary tool for analyzing macrophages is the CD11b-DTR system. However, CD11b is a widely expressed marker among multiple immune cell types causing caveat for the interpretation of cell types using this model for understanding liver diseases (**Table 2**) (87).

To follow myeloid cells *in situ* using *in vivo* imaging, flow cytometry or microscopy multiple reporter mouse models have been developed. The promoters from different molecules such as e.g., CD11c, Csfr1, CCR2, MHC-II, or CX3CR1 were used to generate these animal models (88) (**Table 2**). These models have their own limitations according to their expression profile that have been reviewed elsewhere (88) (**Table 2**). Some of the promoters are also utilized to express Cre recombinase (**Table 2**). Crossing these Cre expressing lines with animals carrying a flox-ed gene allows the analyses of the cell specific depletion of the gene of interest. Certainly, the specificity and limitation of the models are determined by the expression pattern of the promoter used for Cre expression (87). Despite the availability of these models, only limited have been exploited for understanding specifically liver fibrosis and NASH (**Table 2**).

Taken together, multiple models are available to answer liver immunological questions. While each of the available models has its own limitation, the combination of these models with each other can still pinpoint important contribution of DCs and macrophages in liver pathology.

THE ROLE OF DENDRITIC CELLS AND MACROPHAGES IN CHRONIC LIVER DISEASES

LIVER FIBROSIS PROGRESSION

Liver fibrosis is a common endpoint of many chronic liver diseases such as viral hepatitis, primary biliary cirrhosis, alcoholic and NASH, or autoimmune liver disorder (96, 97). To investigate liver fibrogenesis, several rodent models have been developed inducing toxic (CCL_4), biliary (bile duct ligation), oxidative (TAA-induced), or metabolic (MCD/methionine choline deficient diet induced) liver damage (96, 97). The MCD diet contains high sucrose and fat (usually 40% sucrose and 10% fat) but lacks the amino acid methionine and the small molecule choline that are essential for hepatic β-oxidation and production/secretion of very low density lipoprotein (VLDL). As a result, lipids are deposited in the liver and steatosis, and NASH develops in these animals (98).

Remarkably, even though the molecular mechanisms leading to hepatic cell death are very different, the process of fibrogenesis and the cellular components involved share common hallmarks. Such common components, that have established their role in liver fibrosis, are the macrophages and the recruited inflammatory monocytes.

Major evidence for the involvement of macrophages in liver fibrosis is demonstrated in *in vivo* depletion studies using the CD11b-DTR system and the clodronate-liposome mediated cell depletion. In CCL_4 induced liver injury, the progression of fibrosis was attenuated in the absence of $CD11b^+$ cells and the number of HSC-derived myofibroblasts was greatly reduced (79). The administration of clodronate liposomes similarly suggested that macrophages are pro-fibrogenic and affect the survival of HSCs via TNF and IL-1 induced NF-kb signaling (84, 99). Liver macrophage populations change during liver injury. One of the major changes is the recruitment of inflammatory monocytes to the injured liver and their differentiation toward tissue macrophages (24, 26, 28, 100). Resident KCs in liver injury rapidly secrete pro-inflammatory cytokines such as IL-1β, TNF, CCL2, and CCL5 resulting in recruitment of multiple immune cells involving monocytes as well. The accumulation of circulating $Ly6C^{hi}$ monocytes within the liver is greatly dependent on CCR2/CCL2 and CCL1/CCR8 axis (100). The monocyte recruiting chemokines, however, not only originate from KCs but also from TLR-activated HSCs (101). Moreover, senescent hepatocytes and NF-kb-inducing kinase (NIK) activation in hepatocytes lead to the release of numerous chemokines (86, 102). These chemokines can influence the migration or activation state of macrophages that in turn induce hepatocyte apoptosis. Accordingly, hepatocyte-specific expression of the NIK *in vivo* triggers massive liver inflammation and hepatocyte apoptosis leading to liver fibrosis (86). Thus, the macrophage–hepatocyte cross-talk seems to greatly influence cell recruitment and the activation state of macrophages, thereby affecting the progression of liver injury. The fact that in the above study KC/macrophage depletion using clodronate reversed NIK-induced damage, also strongly suggests this.

Monocyte recruitment to the injured liver can be observed early within 24 h after the induction of CCL_4 damage (25). These early recruited cells are $CCR9^+$, colocalize and interact with $CCR9^+$ HSCs (27). Furthermore, these monocyte-derived macrophages are characterized as $CD11b^+F4/80^+iNOS^+$ cells that exhibit profibrogenic properties via promoting HSC activation, Th1 cell differentiation, and TGFβ release (25, 26). In addition to this, profibrogenic $Ly6C^{hi}$ macrophages express PDGF, IL-13 and IL-4 that directly act on HSC derived myofibroblasts and induce ECM production (25, 26). Macrophages produce various lectins among them galectin-3 is required for TGFβ mediated myofibroblast activation and matrix production that further underline their profibrogenic capacity (103).

Another chemokine that affects the infiltrating monocytes is the fractalkine receptor (CX3CR1). Fractalkine is released by hepatocytes and HSCs during liver injury. It ensures the survival of infiltrating monocytes and influences their differentiation toward tissue macrophages (25). In the absence of CX3CR1, infiltrating monocytes develop into highly inflammatory macrophages that die early via apoptosis. This perpetuates further inflammation and recruitment of $Ly6C^{hi}$ cells. Additionally, CX3CR1 on KCs increase their IL-10 expression and reduces their TNF and TGFβ (104). Thus, fractalkine represent a negative feedback on the extension of liver inflammation through affecting KCs and the presence and destiny of $Ly6C^{hi}$ cells at least in the murine system. It requires future research to clarify how the changes in monocyte and macrophage subsets observed in mice are reflected in humans.

Regarding the M1–M2 classification of macrophages, during the progression phase of liver fibrosis and during fibrosis resolution, both types of cells are present in the liver side by side (105). Interestingly, based on histological analyses these M1 and M2 macrophages localize near to the fibrotic septa and could

indicate further undiscovered cross-talk among these cells in liver pathology. Of note, transcriptional analyses of macrophages that are present in the resolution phase display a profile that cannot be classified according to the M1/M2 nomenclature (28).

Accumulation of macrophages within the injured liver caused just partially by the recruited monocytes and their differentiation toward tissue macrophages. There is some evidence that local multiplication of resident and monocyte-derived macrophage population contribute to this process. Ki67 staining during CCL_4 mediated liver injury demonstrated the presence of proliferating KCs and monocyte-derived macrophages (28, 62). In most recent study, Listeria infection of the liver resulted in monocyte-derived macrophage proliferation via IL-4 and IL-33 (106). Whether these cytokines are also involved in this process during other types of liver injury and in humans as well remain to be elucidated.

Multiple animal studies reported that the number of dendritic cells, pre-DCs, and pDCs increase during the progression phase of liver fibrosis (76, 107). This raised the assumption that DCs might contribute to fibrosis progression. Using the CD11c-DTR model, it has been demonstrated that $CD11c^+$ cells provide a pro-inflammatory milieu by producing IL-1β and TNF during injury (76). Moreover, isolated cells contribute to HSC survival *in vitro* suggesting a clear profibrogenic capacity of these cells (37). This phenomenon, despite of the relatively broad CD11c expression among other myeloid cells as discussed above (**Tables 1** and **2**), was attributed to DCs.

Another study determined using the same CD11c-DTR system that DC depletion accelerates the development of fibrosis due to their influence on angiogenesis. DCs seem to be the source of the anti-angiogenic VEGF receptor 1 (also known as sFlt-1) and thus influence the bioavailability of VEGF during fibrogenesis (78). Notably, recent study has demonstrated that $VEGF^+$ inflammatory monocytes/monocyte derived macrophages colocalize with newly formed vessels in injured liver and pharmacological inhibition of CCL2 mediated recruitment of inflammatory monocytes reduces fibrosis-induced angiogenesis without affecting fibrosis progression (108). Thus, recruited monocytes/macrophages seem to counterbalance the anti-angiogenic property of DCs during fibrosis progression. Whether classical DCs or pDCs truly contribute to fibrosis progression or play other role during liver injury still remain to be clarified in the future.

LIVER FIBROSIS REGRESSION

During liver fibrosis, the increased production of ECM is accompanied by high expression of MMPs and the presence of collagenase activity, suggesting alterations and adjustments in the fibrotic ECM. In fact, the fibrotic ECM seems to be different biochemically than ECM produced during a steady state turnover (109). In lung fibrosis, the pathological ECM activates fibroblastic cells to build further matrix indicating a positive cross-talk between fibroblast and matrix components (109). Also during liver fibrosis, heavily cross-linked, modified ECM could be identified (110); however, it remains to be elucidated whether similar regulatory loop as in the lung operates in liver fibrosis as well.

Importantly, after removal of the noxious agents causing liver damage, fibrotic scars degrade and normal liver architecture can be restored. This process is called resolution. While this functions well in various animal models, in humans this seems to be a point of no return where fibrosis and cirrhosis progresses nonetheless (97).

In resolution, the role of macrophages has been demonstrated in multiple animal studies. Depletion of $CD11b^+$ cells during fibrosis progression, as above discussed, reduced scarring while during fibrosis resolution led to a failure in matrix degradation (79, 80). This strongly suggests the dominant presence of two functionally different macrophage populations. According to this, Ramachandran et al. have identified a subset of $Ly6C^{low}$ "restorative" macrophages during resolution (28). These cells originated from $Ly6C^{hi}$ recruited monocytes expressed MMPs including MMP9, MMP12, and phagocytosis related genes. Importantly, based on gene expression profiling, they could not be fit in the M1/M2 macrophage classification. Moreover, phagocytosis of liposomes or cellular debris by liver macrophages could recapitulate this type of restorative phenotype (28). In addition to this, recent study demonstrated that scar associated myeloid cells attract endothelial cells to the scar tissue via VEGF and that genetic ablation of VEGF in myeloid cells resulted in the increase of MMP2 and MMP13 and decrease of TIMP1 in the liver. While macrophages have not been unequivocally identified as myeloid cells in this study, the results indicate that the myeloid cells induced angiogenesis gears the balance toward fibrolysis (111). This is in line with recent findings that demonstrated that VEGF signaling plays key role during liver fibrosis resolution. Anti-VEGF antibody treatment during resolution led to impaired tissue repair. Mechanistically, VEGF regulated endothelial permeability, monocyte recruitment, and affected the CXCL9 and MMP13 expression of scar-associated macrophages. Importantly, depletion of $Cfsr1^+$ cells (including macrophages, monocytes, and DCs **Table 2**) impaired fibrosis resolution (112).

Based on these findings, macrophages can be grouped in profibrogenic and restorative macrophage populations beyond the M1/M2 scheme, a classification that might be much more beneficial for finding new targets for fibrosis therapy. However, multiple open questions remain concerning the balance of the heterogeneous population in liver diseases and the relation to each other. One molecule could provide a better understanding to the problem, the chemokine CX3CL1. Ramachandran et al. showed a higher expression of CX3CR1 within the restorative macrophage population, then in the profibrotic subset (28). Consistent with these findings, HSC and hepatocyte-derived fractalkine led to the induction of Arginase 1 in a mixed Kupffer-cell/macrophage cell population, a marker that has been associated with the fibrolytic macrophage subset (28, 104). Thus, an intriguing possibility is the progressive class switch between macrophage populations during fibrosis progression and regression. This possibility is underlined by the fact that the overall number of profibrogenic $Ly6C^{hi}$ macrophages strongly decreases in resolution despite the presence of their strong proliferation activity at early time points of fibrosis regression. At the same time, the number of $Ly6C^{lo}$ macrophages increases (28). Along this line, blocking CCL2 dependent liver infiltration by $Ly6C^{hi}$ monocytes during fibrosis regression leads to a higher relative amount of $Ly6C^{lo}$ macrophages (113). Moreover, the $Ly6C^{lo}$ macrophages could be shown to be postphagocytic and seem to appear in the phase of reduced hepatocyte death, further supporting the switch concept (28). It remains to be clarified

in the future how the macrophage populations interact and relate to each other. Similarly as the murine restorative macrophages in humans, this population is likely represented by the $CD14^+$ $CD16^+$ cells (24, 26). They display phagocytic activity but as opposite to the murine cells express a variety of pro-inflammatory and pro-fibrogenic molecules as well.

Besides macrophages, DCs have also been implicated in liver fibrosis resolution. Jiao et al. have demonstrated that depletion of $CD11c^+$ cells leads to delay in fibrosis resolution and delayed clearance of activated HSCs. To more precisely pinpoint DCs in this process, adoptive transfer of purified DCs or expansion of endogenous DCs using FLT3L could accelerate regression. Moreover, DCs were the source of MMP9 and therefore seem to complement restorative macrophages in this process (75).

NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome that includes hypertension, hyperlipidemia, insulin resistance, and visceral adiposity, and shows a worldwide increasing tendency among chronic liver diseases (114, 115). In most cases, the liver steatosis is mild. However, up to one-fifth of the cases progresses toward NASH that is characterized by intrahepatic inflammation, increased steatosis with hepatocellular ballooning, and often accompanied by progressive fibrosis (114, 115). NASH is prone to the development of cirrhosis and liver cancer (115). While the precise cellular and molecular mechanisms of NASH are not yet fully understood, multiple studies have investigated macrophages and DCs in this disease.

Similarly as during liver fibrogenesis, in NASH, the two main components that show alterations are the response of macrophages/KCs and the recruited inflammatory monocytes. The key role of macrophages/KCs in NASH has been demonstrated in studies where these cells were specifically depleted using gadolinium chloride or clodronate liposomes (81–83, 85). In the absence of KCs, the steatohepatitis was markedly reduced. In addition to this, KCs display an M1 TNF expressing pro-inflammatory phenotype and increase triglyceride accumulation, decrease fatty acid oxidation and insulin responsiveness of hepatocytes (82, 83). KC-derived TNF production seems to be central in NASH development, as silencing liver TNF or using TNFR1/2 deficient animals attenuate liver steatosis compared with control wild-type animals (85, 116).

Multiple triggers have been identified for KC activation and for the induction of their pro-inflammatory cytokine production in NASH. TLR4 deficient animals showed reduced liver damage and KC depletion prevented the increase in TLR4 expression during MCD diet (81). Bacterial product induced KC activation is in accordance with clinical data that demonstrate bacterial translocation in NASH patients (117). Notably, TLR4 can be triggered not only by LPS but also by free fatty acids and high mobility group box-1 protein (HMGB1) that is increased in obesity and during hepatocyte injury. Not only LPS but also translocated nucleic acids have been implicated in the development of NASH via triggering TLR9 mediated KC activation and IL-1β release (118).

Lipidomics and mass spectrometry profiling revealed that KCs accumulate toxic lipids due to the dysregulation of lipid metabolism during high fat diet. Moreover, these lipid-loaded KCs produce pro-inflammatory cytokines and chemokines (119). The balance between the M1 and M2 type of KCs seem to be a key for NASH progression. Mice fed with high-fat diet displayed a predominant M2 KC polarization, the apoptosis of M1 KCs and resistance to hepatocyte steatosis. *In vitro* experiments demonstrated that M2 macrophages release IL-10 that in return increase the sensitivity of M1 macrophages to undergo apoptosis (120).

The other hallmark of NASH is the increased monocyte recruitment to the injured liver. Activated KCs upregulate their MCP-1 expression that is the major chemokine involved in the recruitment of $Ly6C^{hi}$ cells. These $Ly6C^{hi}$ cells are pro-inflammatory and further perturb hepatic inflammation (85). Consequently, CCR2 deficient animals show decreased steatosis (113, 121). On the other hand, CCR2 signaling, when MCD diet is replaced with control diet, counteracts tissue resolution by perpetuating inflammation (113). This is a similar phenomenon as observed in fibrosis resolution (113).

Non-alcoholic steatohepatitis is associated with increased number of hepatic DCs identified by $MHCII^+$ $CD11c^+$ cells (77). Depletion of these cells using CD11c-DTR mouse model exacerbates hepatic inflammation whereas during the resolution phase delays the reconstitution of normal tissue homeostasis. Importantly, these cells take up apoptotic cells, inhibit TLR expression, T cell expansion, and cytokine production by innate cells (77). This strongly suggests DCs as an important negative regulator of NASH inflammation. As opposite to this, another study has classified $CD11c^+$ cells during MCD-diet based on their lipid content (43). It remains to be clarified whether the tolerogenic LL-DC (low lipid DC) population is equivalent with the same immunoregulatory DCs in NASH as described by Henning et al. Of note, based on the surface marker expression profile of HL-DCs and LL-DCs, they rather seemed to be a part of a heterogeneous population, despite that all cells expressed various level of CD11c (43).

SUMMARY AND CONCLUSION

Taken together, the liver represents a unique immunological niche within the body. Its parenchymal and non-parenchymal cells guard its tolerogenic and suppressive microenvironment while supporting its sentinel task of the portal and systemic circulation (**Figure 1**). Most liver injuries trigger the activation of resident KC/macrophage population that rapidly releases pro-inflammatory mediators such as TNF and IL-1β. This is followed by a chain of events that seem to be commonly shared by many injuries causing NASH and leading to liver fibrosis. The response involves the alterations within the myeloid cell composition primarily affecting macrophages. Importantly, other immune cells such as DCs, neutrophils, innate cells, and activated T cell are also recruited to the injured liver and play various roles in disease progression (6, 97). The exact role of liver DCs during chronic liver injury is yet to be determined. Nevertheless, they seem to be similarly pro-inflammatory as the $Ly6C^{hi}$ recruited monocytes. This feature is shared with infectious liver diseases; thus, it supposes to induce liver protecting immunity (2, 7). During chronic liver diseases, the overwhelming presence of pro-inflammatory immune cells together with liver damaging noxious agents eventually lead to extensive cell death and scar formation, a common outcome for chronic liver disorders. While KC activation alarms other immune

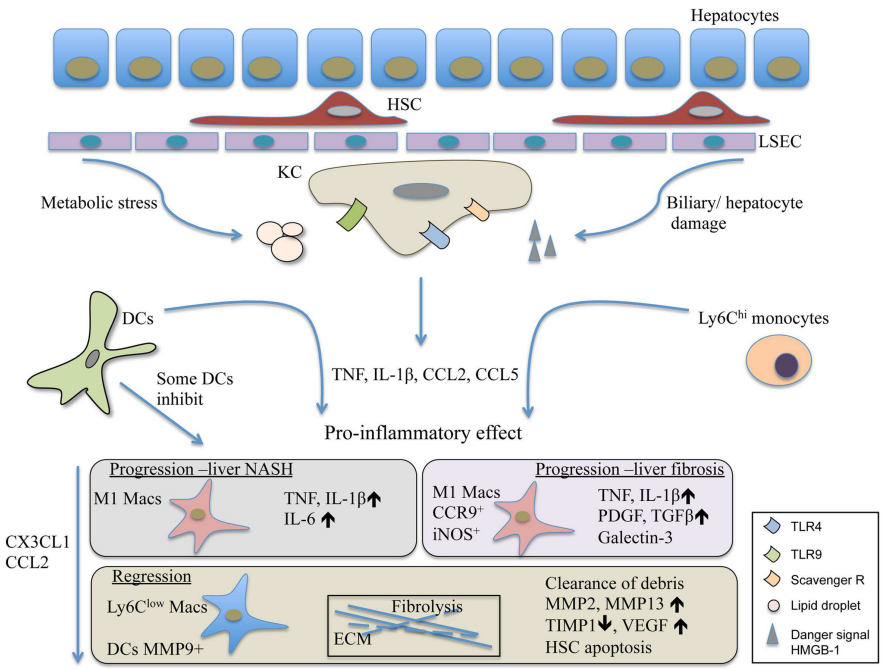

FIGURE 1 | The contribution of DCs and macrophages to the pathomechanism of liver fibrosis and NASH. Liver injury triggers the activation of Kuppfer cells, the resident macrophage population of the liver. Their activation leads to the release of inflammatory mediators and chemokines such as TNF, IL-1β, and CCL2. This is followed by the recruitment of various immune cells involving inflammatory monocytes and DCs. The Ly6C^{hi} monocytes differentiate into M1 CCR9^{+}iNOS^{+} macrophages, and together with DCs in the progression phase of liver injury, act in a pro-inflammatory manner and perpetuate inflammation. Some DCs, possibly the LL-DCs, seem to inhibit liver steatohepatitis and protect liver damage. In resolution, the Ly6C^{low} restorative macrophages together with MMP9^{+} DCs promote fibrolysis and the restoration of normal tissue architecture. HMGB-1, high mobility group box-1 protein; HSC, hepatic stellate cells; KC, Kupffer cells; LL-DC, low lipid containing DCs; LSEC, liver sinusoidal endothelial cells.

cells to travel to the liver, it influences metabolic processes and survival of hepatocytes. During disease progression, Ly6C^{hi} cells seem to develop into Ly6C^{lo} restorative macrophages. These cells, if the harmful agent vanishes, lead to resolution and can restore normal tissue architecture (**Figure 1**). Especially, in this process, DCs are complementing the macrophage population. In infection, recent report demonstrated that necroptosis of KCs was necessary to induce the Th2 mediated tissue repair (106) that remains to be tested to affect fibrosis resolution in the future. Equally important is the more detailed understanding of the factors involved in the switch from the pro-inflammatory to the restorative macrophage population.

Despite of the significant amount of data available in mice, we have just limited understanding about the course of events in human liver diseases. It will need future studies to analyze DC, monocyte, and macrophage populations within human liver samples not only phenotypically and functionally but also on genomic level in comparison with their murine counterparts. This can lead to better understanding of liver diseases but also for identifying novel therapeutic targets. A promising clinical perspective is to target chemokines in the early phase of the liver response to avoid inflammatory cell recruitment and further inflammation. One possibility is affecting the CCL2 axis. Currently, Cenicriviroc, an inhibitor of CCR2, is tested (Centaur study, phase 2 clinical trial, NCT:022117475) to attenuate fibrosis progression in NASH patients. Along this line, other chemokines that could affect the differentiation of monocytes to inflammatory macrophages could be a possible target in the future. Additionally, DCs and restorative macrophages could become novel objectives for inducing fibrolysis and reversing liver damage. Notably, autologous transfer of expanded mononuclear cells to chronic viral hepatitis-associated fibrotic patients showed improved outcome as indicated by reduced Child-Pugh score (122), suggesting a great potential of myeloid cell transfer-based therapeutic procedures in the future.

AUTHOR CONTRIBUTIONS

CE, NK contributed to the writing of the manuscript, prepared the tables. MK supervised the students and critically read the manuscript. VL-K developed the concept of the manuscript, supervised, and wrote the manuscript.

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REFERENCES

1. Ohtani O, Ohtani Y. Lymph circulation in the liver. *Anat Rec (Hoboken)* (2008) **291**:643–52. doi:10.1002/ar.20681
2. Jenne CN, Kubes P. Immune surveillance by the liver. *Nat Immunol* (2013) **14**:996–1006. doi:10.1038/ni.2691
3. Racanelli V, Rehermann B. The liver as an immunological organ. *Hepatology* (2006) **43**:S54–62. doi:10.1002/hep.21060

4. Winau F, Quack C, Darmoise A, Kaufmann SH. Starring stellate cells in liver immunology. *Curr Opin Immunol* (2008) **20**:68–74. doi:10.1016/j.coi.2007.10.006
5. Puche JE, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. *Compr Physiol* (2013) **3**:1473–92. doi:10.1002/cphy.c120035
6. Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. *Nat Rev Immunol* (2010) **10**:753–66. doi:10.1038/nri2858
7. Crispe IN. Immune tolerance in liver disease. *Hepatology* (2014) **60**:2109–17. doi:10.1002/hep.27254
8. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* (2007) **449**:419–26. doi:10.1038/nature06175
9. Hopp AK, Rupp A, Lukacs-Kornek V. Self-antigen presentation by dendritic cells in autoimmunity. *Front Immunol* (2014) **5**:55. doi:10.3389/fimmu.2014.00055
10. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* (2009) **206**:3115–30. doi:10.1084/jem.20091756
11. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* (2012) **13**:888–99. doi:10.1038/ni.2370
12. Yoneyama H, Narumi S, Zhang Y, Murai M, Baggiolini M, Lanzavecchia A, et al. Pivotal role of dendritic cell-derived CXCL10 in the retention of T helper cell 1 lymphocytes in secondary lymph nodes. *J Exp Med* (2002) **195**:1257–66. doi:10.1084/jem.20011983
13. Barbier L, Tay SS, McGuffog C, Triccas JA, McCaughan GW, Bowen DG, et al. Two lymph nodes draining the mouse liver are the preferential site of DC migration and T cell activation. *J Hepatol* (2012) **57**:352–8. doi:10.1016/j.jhep.2012.03.023
14. Matsuno K, Kudo S, Ezaki T. The liver sinusoids as a specialized site for blood-lymph translocation of rat dendritic cells. *Adv Exp Med Biol* (1997) **417**:77–81. doi:10.1007/978-1-4757-9966-8_13
15. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**:571–8. doi:10.1038/nri3712
16. Bosma BM, Metselaar HJ, Mancham S, Boor PP, Kusters JG, Kazemier G, et al. Characterization of human liver dendritic cells in liver grafts and perfusates. *Liver Transpl* (2006) **12**:384–93. doi:10.1002/lt.20659
17. Kelly A, Fahey R, Fletcher JM, Keogh C, Carroll AG, Siddachari R, et al. CD141(+) myeloid dendritic cells are enriched in healthy human liver. *J Hepatol* (2014) **60**:135–42. doi:10.1016/j.jhep.2013.08.007
18. Liu K, Nussenzweig MC. Origin and development of dendritic cells. *Immunol Rev* (2010) **234**:45–54. doi:10.1111/j.0105-2896.2009.00879.x
19. Guo S, Yang C, Mei F, Wu S, Luo N, Fei L, et al. Down-regulation of Z39Ig on macrophages by IFN-gamma in patients with chronic HBV infection. *Clin Immunol* (2010) **136**:282–91. doi:10.1016/j.clim.2010.03.007
20. Nascimento M, Huang SC, Smith A, Everts B, Lam W, Bassity E, et al. Ly6Chi monocyte recruitment is responsible for Th2 associated host-protective macrophage accumulation in liver inflammation due to schistosomiasis. *PLoS Pathog* (2014) **10**:e1004282. doi:10.1371/journal.ppat.1004282
21. Zigmond E, Samia-Grinberg S, Pasmanik-Chor M, Brazowski E, Shibolet O, Halpern Z, et al. Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. *J Immunol* (2014) **193**:344–53. doi:10.4049/jimmunol.1400574
22. Strauss O, Dunbar PR, Bartlett A, Phillips A. The immunophenotype of antigen presenting cells of the mononuclear phagocyte system in normal human liver – a systematic review. *J Hepatol* (2015) **62**:458–68. doi:10.1016/j.jhep.2014.10.006
23. Karlmark KR, Weiskirchen R, Zimmermann HW, Gassler N, Ginhoux F, Weber C, et al. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. *Hepatology* (2009) **50**:261–74. doi:10.1002/hep.22950
24. Liaskou E, Zimmermann HW, Li KK, Oo YH, Suresh S, Stamataki Z, et al. Monocyte subsets in human liver disease show distinct phenotypic and functional characteristics. *Hepatology* (2013) **57**:385–98. doi:10.1002/hep.26016
25. Karlmark KR, Zimmermann HW, Roderburg C, Gassler N, Wasmuth HE, Luedde T, et al. The fractalkine receptor CX(3)CR1 protects against liver fibrosis by controlling differentiation and survival of infiltrating hepatic monocytes. *Hepatology* (2010) **52**:1769–82. doi:10.1002/hep.23894
26. Zimmermann HW, Seidler S, Nattermann J, Gassler N, Hellerbrand C, Zernecke A, et al. Functional contribution of elevated circulating and hepatic non-classical CD14CD16 monocytes to inflammation and human liver fibrosis. *PLoS One* (2010) **5**:e11049. doi:10.1371/journal.pone.0011049
27. Chu PS, Nakamoto N, Ebinuma H, Usui S, Saeki K, Matsumoto A, et al. C-C motif chemokine receptor 9 positive macrophages activate hepatic stellate cells and promote liver fibrosis in mice. *Hepatology* (2013) **58**:337–50. doi:10.1002/hep.26351
28. Ramachandran P, Pellicoro A, Vernon MA, Boulter L, Aucott RL, Ali A, et al. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. *Proc Natl Acad Sci U S A* (2012) **109**:E3186–95. doi:10.1073/pnas.1119964109
29. Liu K, Waskow C, Liu X, Yao K, Hoh J, Nussenzweig M. Origin of dendritic cells in peripheral lymphoid organs of mice. *Nat Immunol* (2007) **8**:578–83. doi:10.1038/ni1462
30. Liu K, Victora GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, et al. In vivo analysis of dendritic cell development and homeostasis. *Science* (2009) **324**:392–7. doi:10.1126/science.1170540
31. Thomson AW, Geller DA, Gandhi C, Murase N, Demetris AJ, Beer-Stolz D. Hepatic antigen-presenting cells and regulation of liver transplant outcome. *Immunol Res* (2011) **50**:221–7. doi:10.1007/s12026-011-8223-0
32. Lukacs-Kornek V, Schuppan D. Dendritic cells in liver injury and fibrosis: shortcomings and promises. *J Hepatol* (2013) **59**:1124–6. doi:10.1016/j.jhep.2013.05.033
33. Xia S, Guo Z, Xu X, Yi H, Wang Q, Cao X. Hepatic microenvironment programs hematopoietic progenitor differentiation into regulatory dendritic cells, maintaining liver tolerance. *Blood* (2008) **112**:3175–85. doi:10.1182/blood-2008-05-159921
34. Kudo S, Matsuno K, Ezaki T, Ogawa M. A novel migration pathway for rat dendritic cells from the blood: hepatic sinusoids-lymph translocation. *J Exp Med* (1997) **185**:777–84. doi:10.1084/jem.185.4.777
35. Khanna A, Morelli AE, Zhong C, Takayama T, Lu L, Thomson AW. Effects of liver-derived dendritic cell progenitors on Th1- and Th2-like cytokine responses in vitro and in vivo. *J Immunol* (2000) **164**:1346–54. doi:10.4049/jimmunol.164.3.1346
36. Jinushi M, Takehara T, Tatsumi T, Yamaguchi S, Sakamori R, Hiramatsu N, et al. Natural killer cell and hepatic cell interaction via NKG2A leads to dendritic cell-mediated induction of CD4 CD25 T cells with PD-1-dependent regulatory activities. *Immunology* (2007) **120**:73–82. doi:10.1111/j.1365-2567.2006.02479.x
37. Abe M, Tokita D, Raimondi G, Thomson AW. Endotoxin modulates the capacity of CpG-activated liver myeloid DC to direct Th1-type responses. *Eur J Immunol* (2006) **36**:2483–93. doi:10.1002/eji.200535767
38. Chen Y, Jiang G, Yang HR, Gu X, Wang L, Hsieh CC, et al. Distinct response of liver myeloid dendritic cells to endotoxin is mediated by IL-27. *J Hepatol* (2009) **51**:510–9. doi:10.1016/j.jhep.2009.04.026
39. Mikami Y, Mizuno S, Nakamoto N, Hayashi A, Sujino T, Sato T, et al. Macrophages and dendritic cells emerge in the liver during intestinal inflammation and predispose the liver to inflammation. *PLoS One* (2014) **9**:e84619. doi:10.1371/journal.pone.0084619
40. Lu L, Woo J, Rao AS, Li Y, Watkins SC, Qian S, et al. Propagation of dendritic cell progenitors from normal mouse liver using granulocyte/macrophage colony-stimulating factor and their maturational development in the presence of type-1 collagen. *J Exp Med* (1994) **179**:1823–34. doi:10.1084/jem.179.6.1823
41. Bamboat ZM, Stableford JA, Plitas G, Burt BM, Nguyen HM, Welles AP, et al. Human liver dendritic cells promote T cell hyporesponsiveness. *J Immunol* (2009) **182**:1901–11. doi:10.4049/jimmunol.0803404
42. Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, et al. Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. *Immunity* (2012) **37**:60–73. doi:10.1016/j.immuni.2012.04.012
43. Ibrahim J, Nguyen AH, Rehman A, Ochi A, Jamal M, Graffeo CS, et al. Dendritic cell populations with different concentrations of lipid regulate tolerance and immunity in mouse and human liver. *Gastroenterology* (2012) **143**:1061–72. doi:10.1053/j.gastro.2012.06.003
44. Villadangos JA, Young L. Antigen-presentation properties of plasmacytoid dendritic cells. *Immunity* (2008) **29**:352–61. doi:10.1016/j.immuni.2008.09.002
45. Swiecki M, Gilfillan S, Vermi W, Wang Y, Colonna M. Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and

CD8(+) T cell accrual. *Immunity* (2010) **33**:955–66. doi:10.1016/j.immuni.2010.11.020
46. Tokita D, Sumpter TL, Raimondi G, Zahorchak AF, Wang Z, Nakao A, et al. Poor allostimulatory function of liver plasmacytoid DC is associated with pro-apoptotic activity, dependent on regulatory T cells. *J Hepatol* (2008) **49**:1008–18. doi:10.1016/j.jhep.2008.07.028
47. Goubier A, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, et al. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* (2008) **29**:464–75. doi:10.1016/j.immuni.2008.06.017
48. Castellaneta A, Sumpter TL, Chen L, Tokita D, Thomson AW. NOD2 ligation subverts IFN-alpha production by liver plasmacytoid dendritic cells and inhibits their T cell allostimulatory activity via B7-H1 up-regulation. *J Immunol* (2009) **183**:6922–32. doi:10.4049/jimmunol.0900582
49. Kingham TP, Chaudhry UI, Plitas G, Katz SC, Raab J, Dematteo RP. Murine liver plasmacytoid dendritic cells become potent immunostimulatory cells after Flt-3 ligand expansion. *Hepatology* (2007) **45**:445–54. doi:10.1002/hep.21457
50. Krueger PD, Kim TS, Sung SS, Braciale TJ, Hahn YS. Liver-resident CD103+ dendritic cells prime antiviral CD8+ T cells in situ. *J Immunol* (2015) **194**:3213–22. doi:10.4049/jimmunol.1402622
51. Huang LR, Wohlleber D, Reisinger F, Jenne CN, Cheng RL, Abdullah Z, et al. Intrahepatic myeloid-cell aggregates enable local proliferation of CD8(+) T cells and successful immunotherapy against chronic viral liver infection. *Nat Immunol* (2013) **14**:574–83. doi:10.1038/ni.2573
52. Bowen DG, Zen M, Holz L, Davis T, McCaughan GW, Bertolino P. The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. *J Clin Invest* (2004) **114**:701–12. doi:10.1172/JCI21593
53. Yoneyama H, Matsuno K, Zhang Y, Murai M, Itakura M, Ishikawa S, et al. Regulation by chemokines of circulating dendritic cell precursors, and the formation of portal tract-associated lymphoid tissue, in a granulomatous liver disease. *J Exp Med* (2001) **193**:35–49. doi:10.1084/jem.193.1.35
54. Link A, Hardie DL, Favre S, Britschgi MR, Adams DH, Sixt M, et al. Association of T-zone reticular networks and conduits with ectopic lymphoid tissues in mice and humans. *Am J Pathol* (2011) **178**:1662–75. doi:10.1016/j.ajpath.2010.12.039
55. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**:86–90. doi:10.1126/science.1219179
56. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**:1327–40. doi:10.1016/j.cell.2014.11.023
57. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
58. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* (2013) **38**:792–804. doi:10.1016/j.immuni.2013.04.004
59. Sieweke MH, Allen JE. Beyond stem cells: self-renewal of differentiated macrophages. *Science* (2013) **342**:1242974. doi:10.1126/science.1242974
60. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**:79–91. doi:10.1016/j.immuni.2012.12.001
61. Kinoshita M, Uchida T, Sato A, Nakashima M, Nakashima H, Shono S, et al. Characterization of two F4/80-positive Kupffer cell subsets by their function and phenotype in mice. *J Hepatol* (2010) **53**:903–10. doi:10.1016/j.jhep.2010.04.037
62. Bouwens L, Baekeland M, De Zanger R, Wisse E. Quantitation, tissue distribution and proliferation kinetics of Kupffer cells in normal rat liver. *Hepatology* (1986) **6**:718–22. doi:10.1002/hep.1840060430
63. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol* (2013) **14**:986–95. doi:10.1038/ni.2705
64. Kuniyasu Y, Marfani SM, Inayat IB, Sheikh SZ, Mehal WZ. Kupffer cells required for high affinity peptide-induced deletion, not retention, of activated CD8+ T cells by mouse liver. *Hepatology* (2004) **39**:1017–27. doi:10.1002/hep.20153
65. Wiegard C, Frenzel C, Herkel J, Kallen KJ, Schmitt E, Lohse AW. Murine liver antigen presenting cells control suppressor activity of CD4+CD25+ regulatory T cells. *Hepatology* (2005) **42**:193–9. doi:10.1002/hep.20756
66. Breous E, Somanathan S, Vandenberghe LH, Wilson JM. Hepatic regulatory T cells and Kupffer cells are crucial mediators of systemic T cell tolerance to antigens targeting murine liver. *Hepatology* (2009) **50**:612–21. doi:10.1002/hep.23043
67. Lee WY, Moriarty TJ, Wong CH, Zhou H, Strieter RM, Van Rooijen N, et al. An intravascular immune response to Borrelia burgdorferi involves Kupffer cells and iNKT cells. *Nat Immunol* (2010) **11**:295–302. doi:10.1038/ni.1855
68. Beattie L, Phillips R, Brown N, Owens BM, Chauhan N, Dalton JE, et al. Interferon regulatory factor 7 contributes to the control of Leishmania donovani in the mouse liver. *Infect Immun* (2011) **79**:1057–66. doi:10.1128/IAI.00633-10
69. Polakos NK, Cornejo JC, Murray DA, Wright KO, Treanor JJ, Crispe IN, et al. Kupffer cell-dependent hepatitis occurs during influenza infection. *Am J Pathol* (2006) **168**:1169–78. doi:10.2353/ajpath.2006.050875
70. Tay SS, Wong YC, Roediger B, Sierro F, Lu B, McDonald DM, et al. Intrahepatic activation of naive CD4+ T cells by liver-resident phagocytic cells. *J Immunol* (2014) **193**:2087–95. doi:10.4049/jimmunol.1400037
71. Ganz T. Macrophages and systemic iron homeostasis. *J Innate Immun* (2012) **4**:446–53. doi:10.1159/000336423
72. Uwatoku R, Suematsu M, Ezaki T, Saiki T, Tsuiji M, Irimura T, et al. Kupffer cell-mediated recruitment of rat dendritic cells to the liver: roles of N-acetylgalactosamine-specific sugar receptors. *Gastroenterology* (2001) **121**:1460–72. doi:10.1053/gast.2001.29594
73. Schmieg J, Yang G, Franck RW, Van Rooijen N, Tsuji M. Glycolipid presentation to natural killer T cells differs in an organ-dependent fashion. *Proc Natl Acad Sci U S A* (2005) **102**:1127–32. doi:10.1073/pnas.0408288102
74. Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. *Immunity* (2014) **41**:21–35. doi:10.1016/j.immuni.2014.06.013
75. Jiao J, Sastre D, Fiel MI, Lee UE, Ghiassi-Nejad Z, Ginhoux F, et al. Dendritic cell regulation of carbon tetrachloride-induced murine liver fibrosis regression. *Hepatology* (2012) **55**:244–55. doi:10.1002/hep.24621
76. Connolly MK, Bedrosian AS, Mallen-St Clair J, Mitchell AP, Ibrahim J, Stroud A, et al. In liver fibrosis, dendritic cells govern hepatic inflammation in mice via TNF-alpha. *J Clin Invest* (2009) **119**:3213–25. doi:10.1172/JCI37581
77. Henning JR, Graffeo CS, Rehman A, Fallon NC, Zambirinis CP, Ochi A, et al. Dendritic cells limit fibroinflammatory injury in nonalcoholic steatohepatitis in mice. *Hepatology* (2013) **58**:589–602. doi:10.1002/hep.26267
78. Blois SM, Piccioni F, Freitag N, Tirado-Gonzalez I, Moschansky P, Lloyd R, et al. Dendritic cells regulate angiogenesis associated with liver fibrogenesis. *Angiogenesis* (2014) **17**:119–28. doi:10.1007/s10456-013-9382-5
79. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest* (2005) **115**:56–65. doi:10.1172/JCI22675
80. Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, et al. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J Immunol* (2007) **178**:5288–95. doi:10.4049/jimmunol.178.8.5288
81. Rivera CA, Adegboyega P, Van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* (2007) **47**:571–9. doi:10.1016/j.jhep.2007.04.019
82. Huang W, Metlakunta A, Dedousis N, Zhang P, Sipula I, Dube JJ, et al. Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. *Diabetes* (2010) **59**:347–57. doi:10.2337/db09-0016
83. Lanthier N, Molendi-Coste O, Cani PD, Van Rooijen N, Horsmans Y, Leclercq IA. Kupffer cell depletion prevents but has no therapeutic effect on metabolic and inflammatory changes induced by a high-fat diet. *FASEB J* (2011) **25**:4301–11. doi:10.1096/fj.11-189472
84. Sunami Y, Leithauser F, Gul S, Fiedler K, Guldiken N, Espenlaub S, et al. Hepatic activation of IKK/NFkappaB signaling induces liver fibrosis via macrophage-mediated chronic inflammation. *Hepatology* (2012) **56**:1117–28. doi:10.1002/hep.25711
85. Tosello-Trampont AC, Landes SG, Nguyen V, Novobrantseva TI, Hahn YS. Kuppfer cells trigger nonalcoholic steatohepatitis development in diet-induced mouse model through tumor necrosis factor-alpha production. *J Biol Chem* (2012) **287**:40161–72. doi:10.1074/jbc.M112.417014
86. Shen H, Sheng L, Chen Z, Jiang L, Su H, Yin L, et al. Mouse hepatocyte overexpression of NF-kappaB-inducing kinase (NIK) triggers fatal macrophage-dependent liver injury and fibrosis. *Hepatology* (2014) **60**:2065–76. doi:10.1002/hep.27348

87. Chow A, Brown BD, Merad M. Studying the mononuclear phagocyte system in the molecular age. *Nat Rev Immunol* (2011) **11**:788–98. doi:10.1038/nri3087
88. Hume DA. Applications of myeloid-specific promoters in transgenic mice support in vivo imaging and functional genomics but do not support the concept of distinct macrophage and dendritic cell lineages or roles in immunity. *J Leukoc Biol* (2011) **89**:525–38. doi:10.1189/jlb.0810472
89. Bar-On L, Jung S. Defining in vivo dendritic cell functions using CD11c-DTR transgenic mice. *Methods Mol Biol* (2010) **595**:429–42. doi:10.1007/978-1-60761-421-0_28
90. Torres PF, Slegers TP, Peek R, Van Rooijen N, Van Der Gaag R, Kijlstra A, et al. Changes in cytokine mRNA levels in experimental corneal allografts after local clodronate-liposome treatment. *Invest Ophthalmol Vis Sci* (1999) **40**:3194–201.
91. Hochweller K, Striegler J, Hammerling GJ, Garbi N. A novel CD11c.DTR transgenic mouse for depletion of dendritic cells reveals their requirement for homeostatic proliferation of natural killer cells. *Eur J Immunol* (2008) **38**:2776–83. doi:10.1002/eji.200838659
92. Meredith MM, Liu K, Darrasse-Jeze G, Kamphorst AO, Schreiber HA, Guermonprez P, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J Exp Med* (2012) **209**:1153–65. doi:10.1084/jem.20112675
93. Meredith MM, Liu K, Kamphorst AO, Idoyaga J, Yamane A, Guermonprez P, et al. Zinc finger transcription factor zDC is a negative regulator required to prevent activation of classical dendritic cells in the steady state. *J Exp Med* (2012) **209**:1583–93. doi:10.1084/jem.20121003
94. Satpathy AT, Kc W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med* (2012) **209**:1135–52. doi:10.1084/jem.20120030
95. Schraml BU, Van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* (2013) **154**:843–58. doi:10.1016/j.cell.2013.07.014
96. Liedtke C, Luedde T, Sauerbruch T, Scholten D, Streetz K, Tacke F, et al. Experimental liver fibrosis research: update on animal models, legal issues and translational aspects. *Fibrogenesis Tissue Repair* (2013) **6**:19. doi:10.1186/1755-1536-6-19
97. Pellicoro A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. *Nat Rev Immunol* (2014) **14**:181–94. doi:10.1038/nri3623
98. Takahashi Y, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* (2012) **18**:2300–8. doi:10.3748/wjg.v18.i19.2300
99. Pradere JP, Kluwe J, De Minicis S, Jiao JJ, Gwak GY, Dapito DH, et al. Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. *Hepatology* (2013) **58**:1461–73. doi:10.1002/hep.26429
100. Tacke F, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. *J Hepatol* (2014) **60**:1090–6. doi:10.1016/j.jhep.2013.12.025
101. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* (2007) **13**:1324–32. doi:10.1038/nm1663
102. Irvine KM, Skoien R, Bokil NJ, Melino M, Thomas GP, Loo D, et al. Senescent human hepatocytes express a unique secretory phenotype and promote macrophage migration. *World J Gastroenterol* (2014) **20**:17851–62. doi:10.3748/wjg.v20.i47.17851
103. Henderson NC, Mackinnon AC, Farnworth SL, Poirier F, Russo FP, Iredale JP, et al. Galectin-3 regulates myofibroblast activation and hepatic fibrosis. *Proc Natl Acad Sci U S A* (2006) **103**:5060–5. doi:10.1073/pnas.0511167103
104. Aoyama T, Inokuchi S, Brenner DA, Seki E. CX3CL1-CX3CR1 interaction prevents carbon tetrachloride-induced liver inflammation and fibrosis in mice. *Hepatology* (2010) **52**:1390–400. doi:10.1002/hep.23795
105. Beljaars L, Schippers M, Reker-Smit C, Martinez FO, Helming L, Poelstra K, et al. Hepatic localization of macrophage phenotypes during fibrogenesis and resolution of fibrosis in mice and humans. *Front Immunol* (2014) **5**:430. doi:10.3389/fimmu.2014.00430
106. Bleriot C, Dupuis T, Jouvion G, Eberl G, Disson O, Lecuit M. Liver-resident macrophage necroptosis orchestrates type 1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. *Immunity* (2015) **42**:145–58. doi:10.1016/j.immuni.2014.12.020
107. Bleier JI, Katz SC, Chaudhry UI, Pillarisetty VG, Kingham TP III, Shah AB, et al. Biliary obstruction selectively expands and activates liver myeloid dendritic cells. *J Immunol* (2006) **176**:7189–95. doi:10.4049/jimmunol.176.12.7189
108. Ehling J, Bartneck M, Wei X, Gremse F, Fech V, Mockel D, et al. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut* (2014) **63**:1960–71. doi:10.1136/gutjnl-2013-306294
109. Parker MW, Rossi D, Peterson M, Smith K, Sikstrom K, White ES, et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *J Clin Invest* (2014) **124**:1622–35. doi:10.1172/JCI71386
110. Issa R, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MD, et al. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology* (2004) **126**:1795–808. doi:10.1053/j.gastro.2004.03.009
111. Kantari-Mimoun C, Castells M, Klose R, Meinecke AK, Lemberger UJ, Rautou PE, et al. Resolution of liver fibrosis requires myeloid cell-driven sinusoidal angiogenesis. *Hepatology* (2015). doi:10.1002/hep.27635
112. Yang L, Kwon J, Popov Y, Gajdos GB, Ordog T, Brekken RA, et al. Vascular endothelial growth factor promotes fibrosis resolution and repair in mice. *Gastroenterology* (2014) **146**:1339–1350 e1331. doi:10.1053/j.gastro.2014.01.061
113. Baeck C, Wei X, Bartneck M, Fech V, Heymann F, Gassler N, et al. Pharmacological inhibition of the chemokine C-C motif chemokine ligand 2 (monocyte chemoattractant protein 1) accelerates liver fibrosis regression by suppressing Ly-6C(+) macrophage infiltration in mice. *Hepatology* (2014) **59**:1060–72. doi:10.1002/hep.26783
114. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* (2013) **10**:686–90. doi:10.1038/nrgastro.2013.171
115. Michelotti GA, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nat Rev Gastroenterol Hepatol* (2013) **10**:656–65. doi:10.1038/nrgastro.2013.183
116. Tomita K, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, et al. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* (2006) **55**:415–24. doi:10.1136/gut.2005.071118
117. Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* (2009) **49**:1877–87. doi:10.1002/hep.22848
118. Miura K, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* (2010) **139**:323–334 e327. doi:10.1053/j.gastro.2010.03.052
119. Leroux A, Ferrere G, Godie V, Cailleux F, Renoud ML, Gaudin F, et al. Toxic lipids stored by Kupffer cells correlates with their pro-inflammatory phenotype at an early stage of steatohepatitis. *J Hepatol* (2012) **57**:141–9. doi:10.1016/j.jhep.2012.02.028
120. Wan J, Benkdane M, Teixeira-Clerc F, Bonnafous S, Louvet A, Lafdil F, et al. M2 Kupffer cells promote M1 Kupffer cell apoptosis: a protective mechanism against alcoholic and nonalcoholic fatty liver disease. *Hepatology* (2014) **59**:130–42. doi:10.1002/hep.26607
121. Baeck C, Wehr A, Karlmark KR, Heymann F, Vucur M, Gassler N, et al. Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. *Gut* (2012) **61**:416–26. doi:10.1136/gutjnl-2011-300304
122. Schuppan D, Kim YO. Evolving therapies for liver fibrosis. *J Clin Invest* (2013) **123**:1887–901. doi:10.1172/JCI66028

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Human and mouse mononuclear phagocyte networks: a tale of two species?

Gary Reynolds[1,2] *and Muzlifah Haniffa*[1]*

[1] Human Dendritic Cell Laboratory, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK,
[2] Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

Dendritic cells (DCs), monocytes, and macrophages are a heterogeneous population of mononuclear phagocytes that are involved in antigen processing and presentation to initiate and regulate immune responses to pathogens, vaccines, tumor, and tolerance to self. In addition to their afferent sentinel function, DCs and macrophages are also critical as effectors and coordinators of inflammation and homeostasis in peripheral tissues. Harnessing DCs and macrophages for therapeutic purposes has major implications for infectious disease, vaccination, transplantation, tolerance induction, inflammation, and cancer immunotherapy. There has been a paradigm shift in our understanding of the developmental origin and function of the cellular constituents of the mononuclear phagocyte system. Significant progress has been made in tandem in both human and mouse mononuclear phagocyte biology. This progress has been accelerated by comparative biology analysis between mouse and human, which has proved to be an exceptionally fruitful strategy to harmonize findings across species. Such analyses have provided unexpected insights and facilitated productive reciprocal and iterative processes to inform our understanding of human and mouse mononuclear phagocytes. In this review, we discuss the strategies, power, and utility of comparative biology approaches to integrate recent advances in human and mouse mononuclear phagocyte biology and its potential to drive forward clinical translation of this knowledge. We also present a functional framework on the parallel organization of human and mouse mononuclear phagocyte networks.

Keywords: mononuclear phagocyte system, dendritic cells, macrophages, monocytes, comparative genomics

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***Correspondence:**
Muzlifah Haniffa,
Human Dendritic Cell Laboratory, Institute of Cellular Medicine, The Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, UK
m.a.haniffa@ncl.ac.uk

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Introduction

The mononuclear phagocyte system (MPS) is a branch of the immune system comprising dendritic cells (DCs), macrophages, and monocytes (1–3). The many functions of the MPS include tissue maintenance and healing, innate immunity and pathogen clearance, and the induction of adaptive immune responses (1–3). Manipulating these functions could lead to clinical benefit, such as modulating DCs to develop antigen-specific anti-tumor immunity or suppressing peripheral autoreactive T cell responses in autoimmunity (4, 5). Several factors need to be considered in designing immunotherapy targeting the MPS, including cellular or pathway target choice and the relevant disease and tissue context. Diversity and plasticity of the MPS, two core features that are paramount for directing the quantity and quality of specific immune responses, have frustrated attempts to develop successful focused therapies. The additional variable of local tissue environment, which also heavily influences the

composition and function of resident and infiltrating mononuclear phagocytes (MPs), also requires careful consideration (1–3).

The MPS was conceived in the 1960s by van Furth to encompass a family of phagocytic mononuclear leukocytes regarded as functional variations of monocytes (6). DCs were embraced as members of the MPS several years later (7). The revolutionary discovery that human monocytes and CD34$^+$ hematopoietic stem cells (HSCs) could be differentiated into DC (mo-DC) and macrophage-like (mo-Mac) cells provided a convenient *in vitro* model to study human MP biology (8–10). However, murine studies have demonstrated the independence of many DCs, macrophages, and Langerhans cells (LCs) from blood monocytes questioning the accuracy of human *in vitro* monocyte-derived cells in recapitulating *in vivo* populations (11–16). Conventional DCs arise from HSCs along a lineage that does not go through a monocyte stage and are dependent on the growth factor receptor FLT3 (11). In contrast, the majority of tissue macrophages arise from prenatally seeded precursors that can survive into adulthood and are dependent on CSF1-R (12–16).

The constituents of MPS share overlapping surface markers, which poses a challenge in parsing functionally distinct populations. A rewarding approach to unravel this complexity has been comparative biology analysis (17–28). In essence, comparative biology relies on the concept that core developmental programs and functions such as differential CD4 and CD8 T cell priming, cross-presentation, migration, and cytokine production are likely to be non-redundant and conserved between species. In support of this, around 99% of murine genes have human analogs and around 96% are syntenic, despite the two species having 80 million years of divergent evolution (29). Comparative transcriptomic mapping has revealed conserved gene expression profiles in the two species allowing parallels to be drawn between DC and macrophage subsets (17–28). This approach places comparative analysis as the central fulcrum facilitating the integration of fundamental immunology to fertilize clinical translational strands (**Figure 1**). Integrating this workflow with cutting-edge technologies including single-cell genomics and proteomics approaches has the potential to accelerate discovery in basic MP biology and its clinical applicability (**Figure 1**). Comparative biology has revealed further insights into the origin and function of human and mouse mononuclear phagocyte populations (17–28) and generated new hypotheses to be tested in both species.

The concept of functional specialization as an inherent property imprinted by MP ontogeny and tissue anatomy has been well demonstrated in many murine studies [reviewed in Ref. (1, 3, 30)]. However, the MPS possesses an additional layer of complexity in the form of dynamic mobility, plasticity, and adaptability to tissue/local microenvironment both in steady state and in inflammation (1, 3, 31). These issues have been particularly difficult to dissect in human, where the temporal resolution to observe these kinetics is constrained by snapshot analysis during inflammation and disease without adequate recourse to their onset and evolution (**Figure 2**). Snapshot observations during inflammation may be confounded by temporal variations in MPS composition and function resulting in highly variable biological data. This variability may account for the biological noise inherently observed with outbred humans in contrast to inbred mice in specific pathogen free (SPF) facilities.

Mononuclear phagocytes and their progenitors are in dynamic equilibrium between peripheral tissue, blood, and bone marrow (1, 3, 31, 32). The distinction between MPs within peripheral interstitial tissue and blood can be difficult to establish in highly vascularized organs such as liver and spleen, where large sinusoids are present adjacent to discontinuous endothelial lining that enables greater mobility of leukocytes within these organs. In addition, inflammatory perturbations affect the dynamic equilibrium between tissue, blood, and bone marrow compartments favoring the relative expansion and egress of specific lineages in response to distinct stimuli (33–35). Expansion of monocyte-derived cells dominates the response to inflammatory stimuli in tissue but little is known regarding their fate upon resolution of inflammation (35). Peripheral tissue DCs migrate to the lymph node where they mediate their potent functions upon inflammatory stimuli. Whether they play a prominent role in local tissue immune regulation and how migratory DCs are repopulated during inflammation and its resolution has been poorly characterized.

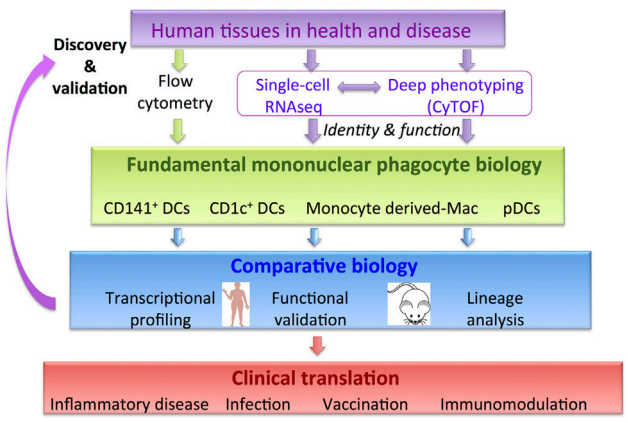

FIGURE 1 | Comparative biology is a validation and discovery tool to pull-through fundamental knowledge in MPS biology to clinical translation. Incorporation of new genomics and proteomics methodologies will accelerate discovery.

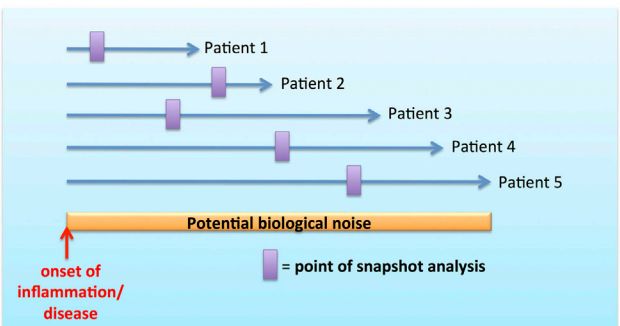

FIGURE 2 | Biological noise with snapshot analysis during temporal course of inflammation and disease.

Comparative Biology to Interrogate Human and Mouse MP Networks

Identifying homology between mice and humans in other hematopoietic cells such as T and B cells has been relatively simple at phenotype and practical levels because of shared lymphocyte surface markers (CD3/CD4/CD8 and CD19, respectively) as well as the relative ease of isolating lymphocytes, which form 90% of human peripheral blood mononuclear cells (c.f. <1% being DCs). Nevertheless, there are functional differences in lymphocytes between the two species, such as differentiation requirements for IL-17 (36) and GM-CSF (37, 38) secreting $CD4^+$ T cells, the specificity of granzyme and FOXP3 expression to define natural Tregs (39), the distinct classes of immunoglobulin (40) and human CD1a, 1b, and 1c-restricted responses to lipid molecules (41). Unfortunately, components of the human and mouse MPS lack overlapping phenotypic markers, hampering initial progress in identifying homologous populations between species.

A range of –omics technologies such as transcriptomics, metabolomics, proteomics, and epigenomics could potentially be employed to assess proximity between species. Of these approaches, transcriptomics is technically most tractable and generates enough complexity to achieve good definition between populations (*n*-dimensions where *n* is the number of genes analyzed) (42, 43). Transcriptome-based comparison of various hematopoietic lineages between human and mouse shows broad conservation but also highlighted specific differences and transcriptional divergence due to gene duplication (43).

Transcriptomics

The hypothesis underlying comparative transcriptomics is that the identified MP populations were present in a shared ancestor and that these same subsets are present in modern animals. Furthermore, despite divergent evolution over time, cells from each subset will have a conserved transcriptomic signature similar to that of its equivalent in the other species. Two approaches are generally used to measuring this similarity: (1) unsupervised hierarchical clustering and principal component analysis (PCA), which assigns samples a point in *n*-dimensional space (*n* corresponding to the number of genes analyzed) and applying a distance metric with greater proximity suggesting a developmental relationship, or (2) supervised assessment of defined transcriptome signature enrichment between populations of interest exemplified by gene set enrichment analysis (GSEA) (44) and its later variations (45).

In hierarchical clustering, the Euclidean distance is calculated between samples. In PCA, the same Euclidean metric is used after the *n*-dimensional data are projected on to the two or three dimensions over which the most variation occurs. This approach has the disadvantages inherent in using large sets of gene data, large number of variables/genes, and high inter-sample variability when testing a limited number of samples. The consistent finding that tissue-specific genes predominate in DC microarray transcriptomes highlights the first point. As a result, microarray data of DC subsets from the same tissue tend to cluster together rather than with their equivalent in blood or another tissue (46). This can be corrected for by techniques such as excluding genes that are differentially expressed between pooled cells from each tissue (and classifying these "tissue-specific") (23, 26) or through using an abbreviated gene panel that is enriched for genes that are known to give good definition between DC subsets (17). An important corollary of this finding is that, while the relative contribution of ontogeny and environment to DC function remains to be determined, the list of genes that define ontogeny is a small fraction of the genes that are modulated by the environment and highlights a potential drawback of using blood DCs as a proxy for tissue DCs.

The use of GSEA derives from large-scale microarray data in which it was recognized that groups of co-regulated functionally linked genes may be more relevant than the few genes that are most significantly differentially regulated but functionally unrelated. This approach is dependent upon an *a priori* understanding of gene function and this can introduce bias. When GSEA has been used in aligning DC subsets between species, a "query signature" is produced that defines the subset of interest. Samples in the test population can then be interrogated for whether they are enriched for this query signature. The underlying analysis is based on the non-parametric goodness-of-fit Kolmogorov–Smirnov test statistic with the reference probability distribution that of the query signature. GSEA and its later variant connectivity map analysis (CMAP) have been successfully used to identify homologous MP populations between species and the developmental origin of human inflammatory DCs (17, 23, 25, 26, 47). Steady state homologous MP populations in human and mouse blood, lymph node, and peripheral tissues are illustrated in **Figure 3**.

Most transcriptomics studies thus far on MPs have involved ensemble or bulk-population analysis. This introduces an inherent bias, as cell populations have to be defined *a priori* based on expression of specific markers. More recently, the application of single-cell RNA-sequencing (sc-RNA-Seq) with unbiased analysis potential has been successfully used to interrogate cellular heterogeneity to

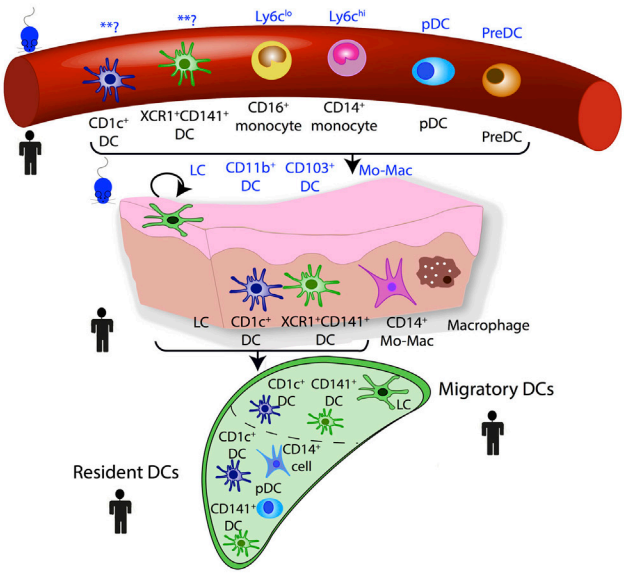

FIGURE 3 | Human and mouse mononuclear phagocyte networks. **? denotes uncertain murine homologs.

uncover new cell populations, functional immune states, and to establish cellular lineage hierarchies and lymphocyte differentiation programs (48–53). These technical advances combined with novel computational approaches have the potential to revolutionize our understanding of MPS biology by unraveling predicted and unexpected functional heterogeneity, which underpins the dynamic repertoire of our immune system in health and disease.

Proteomics

Proteomics analysis has revealed differences in viral sensing pathways between murine splenic DC subsets (54) and identified the murine common monocyte progenitor (cMOP), an intermediate cell-type between the monocyte/macrophage and DC precursor (MDP) and monocyte (55). However, current large-scale proteomics approaches require high cell numbers for robust analysis and are impractical for rare populations, especially from limited human tissue material. Protein expression on a more limited scale has been the mainstay of conventional flow cytometry to define populations and assess MP functions at single-cell resolution. Although the number of parameters that can be analyzed simultaneously is limited (17–18 parameters using commercial instruments), the application of new unbiased probabilistic analysis to define populations could reveal new insights to MP heterogeneity (56). Mass cytometry (CyTOF) provides additional parameters (up to 100) and combined with unbiased population assignment has enormous discovery potential. This combined analysis on mouse myeloid cell populations has revealed far greater population heterogeneity than previously appreciated (57).

Functional Validation

Comparative functional analysis between mouse and human MPs has resulted in variable findings [reviewed in Ref. (30, 58)]. It is unknown if this is due to true biological differences or experimental factors which are not comparable within and between species, including the common use of murine *in vivo* models in contrast to human *in vitro* assays to assess MP functions. Conserved functions are detailed in **Figure 4**.

Lineage Analysis

The power and utility of comparative biology to identify homologous MP populations is beginning to be applied to MP lineage analysis. The recent identification of the successive downstream progenies of human MDP; the Common DC precursor (CDP) and precursor of myeloid DCs (pre-cDCs) exploited the conserved dependency on growth factors and cytokines between human and mouse DC precursors (27, 28). Similarly, comparative analysis suggested the monocyte-origin of human dermal $CD14^+$ cells (25) and inflammatory DCs (47). The preservation of LCs and dermal macrophages in GATA2 and biallelic IRF8 deficiencies show that they are bone marrow independent in the steady state and similar to their murine counterparts, also arise from prenatally seeded precursors (59, 60).

DC, Monocyte, and Macrophage Subsets in Mice and Humans

This approach of using ontogeny and by extension transcription factor dependence to define MPS populations was formalized recently in a proposed nomenclature (61). In this scheme, four adult HSC-derived MP populations are described in mice: two conventional/classical DC subsets (cDC1 and cDC2), plasmacytoid DCs (pDCs), and monocyte-derived cells (61). Both cDCs and pDCs are derived from murine CDP (62, 63). The CDP-derived cells are defined by their dependence on specific transcription factors (TFs): cDC1 are Batf3-dependent, cDC2 are Irf4-dependent, and pDC are E2-2-dependent (61). This definition is unambiguous and avoids using surface markers that can vary between tissues and in inflammation. While the ontogeny approach aids definition of murine populations, it cannot be easily transferred to human DC nomenclature, due to inherent logistical difficulties of human ontogeny studies. However, with the aid of comparative biology approaches, homologous populations between human and mouse MP subsets can be identified and inferences between species on ontogeny and function can be made (**Figures 3** and **4**).

Cell	Human	Common specialisations	Mouse
cDC1	CD141 XCR1 CLEC9A CADM1	• Cross-presentation • IL-12 and IFNλ production • Expression of TLR3 • Generate Th1/Th2 responses	CD103/CD8 XCR1 Clec9A CADM1
cDC2	CD1c CD11b SIRPα	• $CD4^+$ T cell responses • IL-1β, IL-6 and IL-23 production • Expression of all TLRs apart from TLR3(mouse) and TLR9 (human) • Generate Th2/Th17 responses	CD24 CD11b SIRPα
pDC	CD123 CD303 CD304	• Anti-viral responses • IFNα production • Expression of TLR7 and TLR9	SiglecH Bst2 Ly6c
$CD14^+$ monocyte $Ly6c^{hi}$ monocyte	CD14 $CCR2^{hi}$ CD62L	• Inflammatory responses • DC- and macrophage-like differentiation in vitro • High CCR2 expression	$Ly6c^{hi}$ $CCR2^{hi}$
$CD16^+$ monocyte $Ly6c^{lo}$ monocyte	CD16 $CCR2^{lo}$ $CX3CR1^{hi}$	• Patrol endothelium • DC- and macrophage-like differentiation in vitro • Low CCR2 expression	$Ly6c^{lo}$ $CCR2^{lo}$ $CX3CR1^{hi}$
LC	Langerin $CD1a^{+++}$ $CD11c^{lo}$	• Maintain epidermal integrity • Maintain Tregs • Generate Th17 responses	Langerin CD24 CD11b F4/80
Macrophage	DC-SIGN FXIIIa LYVE-1 CD206	• Tissue-resident specialisation conserved between species (e.g. Kupffer cells, microglia, osteoclasts)	F4/80 CD64 MerTK

FIGURE 4 | Conserved specializations between human and mouse mononuclear phagocytes.

cDC1

Phenotype

This subset is identified in mouse by the expression of CD8α in the spleen and CD103 in non-lymphoid tissues (NLT). Its human equivalent in blood and NLT were initially defined by their high expression of CD141 (thrombomodulin, BDCA-3) (19–23). However, this antigen can be upregulated on blood monocytes and expressed promiscuously by other DC subsets in human tissue (23). The cell adhesion molecule CADM1 (NECL2), C-type lectin, CLEC9A (which recognizes damaged cells), and the chemokine receptor XCR1 are expressed on human and mouse cDC1 (19, 22, 64–66). However, CADM1 expression is not restricted to leukocytes and CLEC9A is also expressed on murine DC precursors

(67, 68). Although cDC1 is the only leukocyte expressing XCR1, a commercial antibody against it is currently unavailable. Notably, langerin is expressed on murine but not human cDC1 (23, 69, 70).

Homology

Homology between human (XCR1$^+$CD141$^+$DCs) and mouse (CD8$^+$/CD103$^+$) cDC1 was demonstrated by comparative transcriptomics, phenotype, and functional analyses (17, 19–21, 23, 71). Furthermore, blood and skin CD141$^+$ DCs cluster together separately from CD1c$^+$ DCs, CD14$^+$ and CD16$^+$ monocytes, and pDCs, suggesting that skin XCR1$^+$CD141$^+$ DCs are the tissue equivalents of blood XCR1$^+$CD141$^+$ DCs (23).

Transcription Factors

In addition to Batf3 (72), murine cDC1 differentiation requires Irf8 (73), Id2 (74, 75), and NFIL3 (76). In human, shRNA knockdown of *BATF3* in cord blood HSCs inhibits their differentiation into cDC1 *in vitro* (22). However, cDC1 were detectable in humanized mice reconstituted with *BATF3* knockdown CD34$^+$ HSCs (22). A possible explanation for this seeming contradiction was shown in mice, where in inflammatory conditions (specifically in the presence of IL-12), other members of the Batf family of TFs appear to be able to compensate for loss of Batf3 (77). *ID2* mRNA is expressed at low amounts in human CD34$^+$ HSCs but upregulated during DC differentiation in the presence of GM-CSF and IL-4 (74). Its role is potentially in suppressing B cell differentiation from a common precursor. Definitive evidence for the requirement of ID2 and NFIL3 in cDC1 development in humans is lacking and highlights the potential difficulties of translating TF-based definitions of DC subsets from mice to humans.

Function

The cDC1 subset is thought to be able to efficiently prime CD8$^+$ T cells through functional specializations such as cross-presentation of antigens and the production of IL-12p70 (78–80). This process is important in the induction of tumor immunity and the control of viral and bacterial infections when DCs are not the malignant cells or directly infected. The expression of Clec9A and XCR1 by both murine and human cDC1s supports this notion. cDC1s express a more limited TLR profile than cDC2s with high expression of TLR3 and TLR10 but without TLR4, –5, –7 and –9 (54, 81). TLR3 senses viral dsRNA but the role of TLR10 is currently unknown. Human cDC1s do not produce large amounts of IL-12p70 in response to TLR ligands alone but do following the combination of TLR ligands and CD40-CD40L signaling through activated T cells (71), in common with the finding in mice (82). IFN-λ is produced by murine and human cDC1 upon stimulation with the TLR3 agonist, poly I:C (83).

Murine cDC1s have an advantage over other subsets at cross-presentation of antigens by being able to (1) maintain optimal phagosomal pH for antigen processing (84) and (2) enhance the transfer of proteins from the endosome in to the cytosol so they can be loaded on to MHC Class I (85). This advantage is apparent when assessing cross-presentation of dead cell-derived antigens and upon stimulation with TLR3. However, recent data showed that murine cDC2 are also able to cross-present and cross-prime antigen upon stimulation with R848, a TLR7/8 agonist (86). In human, cDC1 appears to be superior at cross-presenting cell-derived antigen, particularly upon polyI:C stimulation (19–21, 71) and when antigens are delivered to late endosomes and lysosomes (87). However, in common with mice, cDC2 are also able to cross-present soluble antigen and long-peptide particularly upon R848 stimulation (88, 89). The variable findings reported may also be due to type of antigens used in the cross-presentation assays and the validity of comparing murine *in vivo* models with human *in vitro* assays.

In mouse, cDC1 preferentially induce Th1 immune response through IL-12p70 production (90, 91), although Th2 induction has also been reported (92). In human, both cDC1 and cDC2 have been shown to induce Th1 and Th2 responses (93). cDC1s were also shown to promote enhanced Th2 differentiation in response to TSLP in an influenza infection humanized mouse model (94). As most human experiments are performed using blood DCs and *in vitro*, it has been logistically difficult to establish pathogen and tissue-specific effects relevant for driving Th priming *in vivo*.

cDC2

Phenotype

cDC2s in mice are lin$^-$MHCII^{hi}CD11c$^+$CD11b$^+$. However, this fraction also includes monocyte-derived cells and macrophages (95). This is demonstrated by the variable depletion of cells from this fraction in Flt3 or Csf1r KO mice suggesting contamination by Flt3-independent cells (75). This is in contrast to the near complete absence of cDC1 in Flt3 KO mice (74).

Genetic tracing using Clec9A-reporter mouse to identify all CDP-derived cells demonstrated near-complete labeling of cDC1s but variable labeling of CD11b$^+$ DCs in NLT (68). Although this is in keeping with the presence of monocyte-derived cells and macrophages within CD11b$^+$ cells, it does not exclude the possibility of an alternative DC differentiation program that does not undergo a monocyte or CDP intermediate stage. Splenic CD11b$^+$ DCs are divided into an ESAM^{hi} population that requires Notch2-, Flt3, and LTβ-signaling for its development and a monocyte-like ESAM^{lo}Clec12A$^+$CX3CR1$^+$ population that is Flt3-independent and expresses high levels of CD14, TNFα, CCR2, and Lyz2 (96). In murine lung, it has been possible to divide the MHCII$^+$CD11c$^+$CD11b$^+$ fraction into CD11b$^+$CD64$^+$ monocyte-macrophage cells and CD11b$^+$CD24$^+$ cDC2s (24). In murine skin, the MHCII$^+$CD11b$^+$Langerin$^-$ fraction comprises cDC2, monocyte-derived cells, and macrophages (97).

There is evidence that similar heterogeneity may be present within human cDC2. Only 170 genes characterized human cDC2, in comparison to 1020 for cDC1 and 1065 genes for pDCs (23). This limited list of differentially expressed genes predicts heterogeneity within the boundaries of the phenotype parameters used to define human cDC2, specifically a subpopulation derived from or closely related to another mononuclear phagocyte such as CD14$^+$ monocytes.

Human cDC2 (CD1c$^+$ DCs) are defined as lin$^-$MHCII$^+$CD14$^-$ CD16$^-$CD11c$^+$CD1c$^+$ cells, a definition they share with *in vitro* monocyte-derived DCs. Although human peripheral blood and murine cDC2 additionally express CD11b, CX3CR1, and SIRPα, these antigens do not distinguish them from monocyte-derived cells (24, 98). Uniquely in the small intestine, cDC2s co-express CD103 and SIRPα (24, 26). *In vitro* human mo-DCs express

CD206/MMR and CD1a but peripheral blood cDC2 do not (47, 99). However, tissue CD1c$^+$ DCs express CD206 and CD1a (100, 101). In addition, some tissue CD1c$^+$ DCs co-express CD14 particularly during inflammation (47).

Homology

The transcriptional signatures of human blood CD1c$^+$ DCs are enriched with that of mouse spleen CD4$^+$/CD11b$^+$ DCs (17, 23). In NLT, the transcriptional signatures of human small intestine CD103$^+$SIRPα$^+$ DCs and dermal CD1c$^+$ DCs are enriched with that of murine spleen and mesenteric lymph node CD11b$^+$ DCs and dermal CD11b$^+$ DCs, respectively (25, 26). A similar relationship was also observed between murine lung CD11b$^+$ DCs with human blood CD1c$^+$ DCs (24).

Transcription Factors

cDC2 development has been shown to be dependent on the TFs Irf4, PU.1, RelB, and RBPJ (24, 96, 102–108). Irf4 directly supports MHC class II antigen presentation to promote CD4$^+$ T cell responses (109). In humans, CD1c$^+$ DCs express high amounts of IRF4 (24). Interestingly, IRF4 is also required for mo-DC differentiation, suggesting a shared differentiation program between cDC2 and mo-DC. PU.1 interacts with Irf4 but also upregulates Flt3 expression critical for early DC differentiation in mice (110, 111). The PU.1 binding site in the Flt3 promoter is conserved in mice and humans, and so it is thought to be similarly required for DC differentiation in humans (111). Administration of Flt3 results in expansion of DC subsets in lymphoid and non-lymphoid tissue (112). PU.1 mutations in humans and mice are associated with myeloid leukemias (113). Biallelic human IRF8 K108E mutation resulted in complete loss of monocytes, pDCs, cDC1, and cDC2 in the peripheral blood (60). Surprisingly, human autosomal dominant IRF8 T108A mutation results in selective loss of the cDC2 subset and IL-12 production (60). It is now apparent from studies on Irf8^{R294C}(BXH2) and Irf8$^{-/-}$ mice that in addition to cDC1, pDCs and monocytes are also dependent on IRF8 (73, 75, 114–116). However, cDC2 frequency in mice with Irf8$^{-/-}$ and the hypomorphic mutation Irf8^{R294C} are unaffected, in contrast to the findings in humans (73, 75, 114).

Function

The transcriptome of cDC2s is enriched for genes related to antigen processing such as LAMP1, LAMP2, and cathepsins (117). Murine cDC2s have been shown to be able to promote Th17, Th2, and regulatory T cell responses depending upon the pathogen and antigen stimulus (24, 108, 118–120). This may be a consequence of their innate plasticity but could also relate to unresolved heterogeneity within murine cDC2. In human, cDC2 have been shown to induce Th17 differentiation (24).

Both human and mouse cDC2 share many transcriptional and functional similarities with monocyte-derived cells (24, 25, 47, 97). Both cDC2 and mo-DC are capable of promoting naïve CD4$^+$ and CD8$^+$ T cell proliferation and in mice cDC2 appear to be superior at trafficking to lymph nodes (97, 98), leading to the hypothesis that mo-DCs specialize in activating tissue-tropic T cells. Mo-DCs also produce higher levels of monocyte-attracting chemokines (CCL2, CCL7, CCL12) than cDC2s (98). Human blood cDC2 have a TLR expression profile that is close to murine lymphoid cDC2 with significantly higher levels of TLRs 2, 4, and 5 than other DC subsets (81), a profile it also shares with *in vitro* mo-DCs (121). The pathogenic role of cDC2 in human disease is not clear but they have been shown to accumulate in conditions such as RA (122), chronic kidney disease (123), and atopic airway inflammation (124), although their distinction from inflammatory mo-DCs is unclear. Human cDC2 are also implicated in the accumulation of CD103$^+$CD8$^+$ mucosal T cells in the lung and promote fibrosis in the kidney through production of TGFβ (123, 125). Finally, human and mouse cDC2 share a similar cytokine production profile which includes IL-6, IL-23, and IL-1β (24, 81, 126, 127). In addition, unlike murine cDC2, human blood cDC2 can secrete high amounts of IL-12p70 upon in vitro stimulation with R848 and LPS, which was augmented in the presence of IFNγ and CD40L (89).

Plasmacytoid DCs (pDCs)

Phenotype

Plasmacytoid DCs are specialized IFNα producing cells that were first described in human peripheral blood and tonsil (128–131). In blood, their morphology resembles that of lymphocytes but upon *in vitro* culture with IL-3 and CD40L, they acquire dendrites resembling myeloid DCs (129). pDCs are identified in mice by expression of CD11c^{int}CD11b$^-$B220$^+$ in combination with markers such as SiglecH and CD317 (BST2) to exclude a subset of NK cells and precursors of cDCs (132). In humans, they are identified by expression of CD123, CD303, and CD304. CD123 is the IL-3 receptor alpha chain and is also expressed on precursor cells, basophils, and eosinophils (133, 134). CD303 (BDCA-2) is a C-type lectin that is specifically expressed by human pDCs (135). Functionally, it has a role in antigen capture and when ligated it inhibits IFNα production (136). CD304 (BDCA-4) is uniquely expressed by pDC in peripheral blood but is also expressed by other cells such as endothelial cells (137).

Homology

The relative distance of the pDC transcriptome from other leukocyte subsets and its conservation directly aligns murine and human pDCs (17). However, a subset of murine pDCs also appears to have cDC differentiation potential (138, 139), which has not been observed in human.

Transcription Factors

Plasmacytoid DC development in humans and mice is dependent on the transcription factor E2-2 (140). E2-2 opposes default differentiation of precursors into cDCs and controls expression of a range of pDC-associated TFs, including SpiB, Irf7, and Irf8 (140, 141). In humans, haploinsufficiency of E2-2 results in Pitt-Hopkins syndrome, a condition with a range of features including developmental delay and characteristic facial features but without known clinical immunodeficiency (142). A population of CD45RA$^+$CD123$^+$ cells is present in the blood of patients with Pitt-Hopkins syndrome but these cells fail to express CD303 and have severely reduced expression of IFNα, indicating that loss of E2-2 blocks full pDC differentiation (140). The transcription factor

SpiB is required for IFNα production by pDCs in mice (143). SpiB-knockdown in human CD34$^+$ HSCs inhibits pDC differentiation *in vitro* (144).

Function

Plasmacytoid DCs have a functional program that is well-conserved between mice and humans (145). In contrast to cDCs, pDCs express a narrow range of pattern recognition (146). Both mouse and human pDCs express TLR7 and TLR9 (146). TLR8 is expressed at very low amounts if any by human pDCs (81, 147, 148) and appears to have a different function in mice (146, 149, 150). pDCs in both mice and humans are specialized in the production of IFNα and thought to be important in viral immunity but also human autoimmunity such as SLE (151, 152).

Monocytes and Monocyte-Derived Cells

Phenotype

Two subsets of monocytes exist in mice and can be distinguished by the differential expression of Ly6C, CCR2, and CX3CR1. Similarly in humans, there are two monocyte subsets in peripheral blood identified by expression of CD14 and CD16 (CD14^{++}CD16$^-$ and CD14$^+$CD16$^+$) as well as an intermediate phenotype (CD14^{++}CD16$^+$). In addition to these antigens, human monocytes are also heterogeneous for the expression of the angiopoietin receptor, Tie2, and 6-sulfoLanNAc(Slan), a carbohydrate modification of the P-selectin glycoprotein ligand-1 (PSGL-1) (153, 154).

Homology

Homology between peripheral blood monocyte subsets has been demonstrated by the extensive transcript enrichment between Ly6C^{hi}CX3CR1^{lo} and CD14^{++}CD16$^-$ monocytes and between Ly6C^{lo}CX3CR1^{hi} and CD16$^+$ monocytes (18, 23, 155).

Transcription Factors

The TFs that regulate the sequential differentiation of HSCs into MDP in mice include PU.1, Irf8, and Klf4 [reviewed in Ref. (156)]. PU.1 is required at each developmental bifurcation including HSC maintenance (157) and the generation of early myeloid progenitors (16, 158–160). Similarly in humans, PU.1 is required for monocyte differentiation from CD34$^+$ cord blood precursors (161). In murine monopoiesis, Irf8 and Klf4 act together to skew differentiation toward monocytes by antagonizing the granulocyte-supporting TF C/EBPα (115, 162). Consistent with this, human autosomal recessive Irf8 deficiency results in complete loss of circulating monocytes and DCs in the presence of neutrophilia (60).

The TFs that control cell-fate decisions downstream of MDP are less well defined. In mice, Irf5 and TCFEB are implicated during MDP to CMoP differentiation (55). The TF Nur77 has been implicated in Ly6C^{lo}CX3CR1^{hi} monocyte generation (163).

PU.1 and MafB act antagonistically to support human monocyte differentiation into mo-DC and mo-Mac, respectively *in vitro* (164). Irf4 was also implicated in human *in vitro* mo-DC differentiation (165). Irf5 promotes the differentiation of classical/M1 macrophages from human monocytes *in vitro* (166). In contrast, Irf4 activates transcription of the alternative/M2 macrophage markers in mice (167) and humans (168, 169).

Function

CD14$^+$ human and Ly6C^{hi}CX3CR1^{lo} murine monocytes can exhibit considerable functional plasticity as demonstrated by their acquisition of DC-like and macrophage-like characteristics *in vitro* and *in vivo*. Recent fate mapping studies have demonstrated that monocytes do not contribute to tissue-resident macrophages in the steady state (12, 14, 15), with the notable exception of gut and dermal macrophages (14, 97, 170). However, monocytes can give rise to tissue macrophage-like cells in inflammation (35, 171). Monocytes can also differentiate into DC-like cells in the steady state in mucosal tissues and skin (97, 172). This process is enhanced during inflammation (97, 98, 173), including infections with Leishmania (34), Influenza (174), Trypanosoma (175), Listeria (33), and pulmonary *Aspergillus* (176). Alternatively, rather than DC-like or macrophage-like differentiation, monocytes may remain as tissue monocytes upon extravasation (177).

CD14$^+$CD16$^+$ intermediate and CD16$^+$ non-classical monocytes are expanded in multiple disease, infection, and inflammatory states (178). CD16$^+$ monocytes "patrol" the endothelium *in vivo*, are weak phagocytes, and sense nucleic acids and viruses via TLR7 and 8 receptors (155). Additional heterogeneity has been reported within human monocytes. Tie2$^+$ monocytes are associated with angiogenesis and Slan$^+$CD16$^+$ cells, which are also present in inflamed skin, are potent producers of TNF α, IL-1β, and IL-12 (179, 180). Monocyte-derived dermal CD14$^+$ cells express IL-1α (25) have been shown to induce differentiation of follicular helper T cells (126) and provide direct B cell help (181).

Langerhans Cells

Langerhans cells are located in epidermal surfaces such as skin and are characterized by the presence of cytoplasmic organelles containing Langerin called Birbeck granules (182). The function of these organelles is unclear but their absence does not affect their capacity to process and present antigen (183). LCs form a dynamic network with adjacent keratinocytes and protrude dendrites through tight junctions to pick up antigens that have passed the stratum corneum barrier (184). The easy accessibility of LCs and their functional plasticity has generated significant interest in targeting them for vaccination strategies (185).

In the steady state, LCs are maintained independently of the bone marrow through local self-renewal (186–188). Human LCs can proliferate *in situ* and have been shown to remain donor in origin up to 10 years after limb transplant (189–191). During inflammation, LCs can be replaced by circulating precursors. The identity of the circulating LC precursor remains unclear. In mice, there appears to be two waves of replenishment with monocytes in the first wave giving rise to short-term LCs that retain some monocyte features and an as yet unknown CD34$^+$ HSC-derived precursor that gives rise to long-term LCs (186, 187). In humans, CD1c$^+$ DCs are able to upregulate langerin and CD1a, a phenotype resembling LCs, upon *in vitro* culture with TSLP and TGFβ or GM-CSF and BMP7, but the relevance of this to *in vivo* LC differentiation is uncertain (192, 193). Although human LCs can self-renew locally after BMT, they are replaced by donor-derived cells, even after non-myeloablative transplant conditioning (194–196).

Langerhans cells are developmentally independent of Flt3 but dependent on Csf1r. However, it is IL-34 signaling through Csf1r, rather than Csf1, that is critical for LC development and maintenance (197). IL-34 is also expressed in human skin but the dependence of human LCs on this cytokine remains untested.

Phenotype

Human and murine LCs are CD11c^{lo}, langerin^{hi}, EPCAM^{+}, and also characterized by the presence of cytoplasmic Birbeck granules (198). In human, LCs are additionally CD1a^{hi} and CD1c^{+} (23, 199).

Homology

The homology between LCs in humans and mouse is obvious given their exclusive anatomical occupancy and shared expression of langerin, EPCAM, and presence of Birbeck granules. Comparative transcriptomic analysis of human and mouse LCs has never been performed.

Transcription Factors

Langerhans cell development is dependent on PU.1, Runx3, and Id2, although the latter may be dispensable for bone marrow-derived LCs (74, 188, 200, 201).

Function

Langerhans cells are able to induce different immune responses depending on the context. Depletion of murine LCs can either exacerbate or suppress contact hypersensitivity immune response [reviewed in Ref. (202)]. In a mouse model of graft versus host disease (GVHD), LCs neither primed CD8^{+} T cells nor programed their homing to the epidermis but were required for their effector function *in situ* (203). This is consistent with their inability to cross-present antigen *in vivo* (80, 204), although cross-presentation has been reported using *in vitro* assays (205). In mice, LCs appear to be critical for Th17 response against the yeast form of *Candida albicans* in the epidermis through engagement of Dectin-1 and their subsequent production of IL-6 (206). In humans, failure to generate effective Th17 responses (as a result of a range of mutations in, for example, IL-17RA, IL-17F, STAT1 genes) can result in chronic mucocutaneous candidiasis (CMC) (207). However, it is unclear if immunity against *Candida* infections in the skin in healthy individuals is dependent upon LCs. Notably, human LCs do not appear to express Dectin-1, which is important for *Candida* recognition (208). *In vitro* human LCs appear versatile and are capable of generating Th1, Th2 (209), Th17 (210), Th22 (211), and Treg (212) responses depending on the experimental conditions used.

Macrophages

Macrophages are a diverse population of tissue-resident cells with roles in inflammation, tissue homeostasis, and repair. Macrophage identity and function can be influenced by three variables: (1) resident tissue environment; (2) exposure to activation signals; and (3) ontogeny (monocyte- vs. prenatal precursor-derived) [reviewed in Ref. (3)].

The nomenclature of macrophages is based upon their tissue of origin [for example, Kupffer cells (liver), osteoclasts (bone), and microglia (CNS)]. This is in recognition of the central influence of environment on their phenotype and function. Examples of these functional specializations include breakdown of RBCs (Kupffer cells and splenic macrophages), bone resorption (osteoclasts), gut peristalsis (muscularis macrophages), and neural network development and maintenance (microglia) (213–215). Although macrophages in the vast majority of tissues, except dermis and the lamina propria, are prenatally derived, their preservation into adulthood by self-renewal is variable by site and in the presence of inflammation [(15, 216, 217) and reviewed in Ref. (218)]. The relative preservation of dermal macrophages and LCs in patients lacking circulating blood monocytes and DCs due to heterozygote GATA2 and biallelic IRF8 deficiencies supports a prenatal origin of some human macrophages (59, 60).

Microarray transcriptome analysis has identified several thousand transcripts with greater than twofold difference in expression between macrophages from different sites in mice (219), supporting unique local microenvironment-related characteristics. These tissue specific transcripts are more prominent within macrophages than DCs (219) and may reflect the tissue-resident nature of macrophages. The impact and underlying mechanisms of environmental regulation on macrophages was elegantly demonstrated by the unique epigenetic modulation of macrophage in distinct tissues and the ability of macrophages from one environment to develop the characteristics of their counterparts in another tissue (220, 221).

Phenotype

Murine macrophages express the antigens CD11b, CD68, CSF1R, and F4/80 (215). With the exception of F4/80 which is predominantly expressed on eosinophils (222), these antigens are also expressed on human macrophages (223). Furthermore, human alveolar macrophages were shown to express many antigens, which are conserved at transcript level with murine bone marrow-derived macrophages (163).

Homology

Comparative analysis between human and mouse macrophage populations has been poorly studied. In skin, homologous monocyte-derived dermal macrophage populations have been identified (25) but the murine counterparts of human dermal macrophages containing melanin-granules (melanophages) remain uncertain. While a range of transcriptional analyses of human macrophage populations in health and disease have been performed, comparisons between human tissues and across species have not been rigorously undertaken (224).

Transcription Factors

The transcriptional requirements of murine YS-derived macrophages differ to those of HSC-derived macrophages. YS-derived microglia require PU.1 and Irf8 but are independent of Myb, Id2, Batf3, and Klf4 (2, 12, 225). Consistent with macrophage tissue specializations, additional TFs such NFATc1 and Spi-C have been shown to be required for osteoclasts and splenic and bone marrow macrophage differentiation, respectively (226–228).

Function

The M1/M2 paradigm has been described to model the diverse programs of macrophage activation but has largely relied on

in vitro generated macrophages. This has provided a useful tool to examine macrophage activation in the absence of tissue-specific effects. More recently, a spectrum of responses, with M1 and M2 being two poles of a continuum that is transcriptionally apparent, were identified (229). It is unclear how closely human and murine macrophages are aligned in response to a similar range of stimuli. There are inter-species differences in the response to a single stimulus (LPS) between human and mouse *in vitro* derived macrophages; INOS transcript is preferentially induced in mouse but human macrophages characteristically upregulate CCL20, CXCL13, IL-7R, P2RX7, and STAT4 (230).

Mononuclear Phagocytes in Inflammation

Classical Ly6C^{hi}CX3CR1^{lo} monocytes infiltrate inflamed tissues where they can acquire either DC or macrophage properties (33, 231). This *in vivo* process (thought to be analogous to *in vitro* mo-Mac and mo-DC differentiation) can be influenced by local microbiota (97, 98, 170, 171). In infection and disease, monocyte-derived cells accumulate in greater numbers in a broad range of tissues [reviewed in Ref. (232)]. In many such models of infection, they are non-redundant and required for clearance of pathogens by promoting protective Th1 and Th17 responses (34, 233, 234). This suggests that despite shared functions with resident conventional DCs, there are important differences that require the presence of monocyte-derived cells to overcome infection. In murine experimental autoimmune encephalomyelitis, monocytes infiltrate the CNS but are not long-lived and following resolution do not contribute to the microglial pool (231). Analysis of murine Kupffer cells suggests functional heterogeneity between resident and recruited populations (235).

Snapshot analysis of inflamed human tissue similarly reveals additional subsets that are not present in health [(47, 99, 179, 236, 237) and reviewed in Ref. (31)]. These include inflammatory dendritic epidermal cells (IDECs) found in atopic dermatitis, TNF, and iNOS producing DCs (Tip DCs) and slan DCs, found in psoriasis (99, 179, 236, 237). In rheumatoid arthritis synovial fluid and malignant ascites, there is an accumulation of cells that express overlapping markers with blood CD1c^{+} DCs but additionally express CD1a, CD206, SIRPα, and CD14 (47). Monocytes can acquire DC characteristics when cultured with *ex vivo* GM-CSF-primed synovial T cells, which potentially suggests a mechanism for their generation (238). Histiocytes are pathological MPs expressing CD68 and CD163. It is unknown if these cells, often found in granulomas, arise from resident macrophages or are monocyte-derived. Further studies are required to establish the *in vivo* differentiation requirements of inflammatory MP populations and how they contribute to disease.

Conclusion

In this review, we have discussed the parallel organization of the MPS between humans and mice. We demonstrate the use of comparative biology approaches as both a validation and discovery tool to dissect the development and functional heterogeneity of mononuclear phagocytes in a reciprocal manner across the two species. The incorporation of high-dimensional unbiased single-cell genomics and proteomics technologies will facilitate the interrogation of functionally relevant populations with indiscrete phenotypes and validate current definitions of cell-types based on limited antigen expression profile particularly during inflammation. This combined strategy will accelerate the translation of fundamental MPS biology to clinical benefit through enhanced understanding of the pathomechanisms of disease and facilitate the development of novel approaches in vaccination and cancer immunotherapy.

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References

1. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* (2013) **31**:563–604. doi:10.1146/annurev-immunol-020711-074950
2. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol* (2014) **14**:392–404. doi:10.1038/nri3671
3. Varol C, Mildner A, Jung S. Macrophages: development and tissue specialization. *Annu Rev Immunol* (2015) **33**:643–75. doi:10.1146/annurev-immunol-032414-112220
4. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* (1998) **392**:245–52. doi:10.1038/32588
5. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* (2007) **449**:419–26. doi:10.1038/nature06175
6. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ* (1972) **46**:845–52.
7. van Furth R. Current view on the mononuclear phagocyte system. *Immunobiology* (1982) **161**:178–85. doi:10.1016/S0171-2985(82)80072-7
8. Cline MJ. Bactericidal activity of human macrophages: analysis of factors influencing the killing of *Listeria* monocytogenes. *Infect Immun* (1970) **2**:156–61.
9. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* (1994) **179**:1109–18. doi:10.1084/jem.179.4.1109
10. Caux C, Vanbervliet B, Massacrier C, Dezutter-Dambuyant C, de Saint-Vis B, Jacquet C, et al. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. *J Exp Med* (1996) **184**:695–706. doi:10.1084/jem.184.2.695
11. Liu K, Victora GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, et al. In vivo analysis of dendritic cell development and homeostasis. *Science* (2009) **324**:392–7. doi:10.1126/science.1170540
12. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**:86–90. doi:10.1126/science.1219179
13. Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med* (2012) **209**:1167–81. doi:10.1084/jem.20120340
14. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* (2013) **38**:79–91. doi:10.1016/j.immuni.2012.12.001

15. Perdiguero EG, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2014) **518**(7540):547–51. doi:10.1038/nature13989
16. Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, et al. C-myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* (2015) **42**:665–78. doi:10.1016/j.immuni.2015.03.011
17. Robbins SH, Walzer T, Dembele D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* (2008) **9**:R17. doi:10.1186/gb-2008-9-1-r17
18. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. *Blood* (2010) **115**:e10–9. doi:10.1182/blood-2009-07-235028
19. Bachem A, Guttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J Exp Med* (2010) **207**:1273–81. doi:10.1084/jem.20100348
20. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, Ventre E, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**:1283–92. doi:10.1084/jem.20100223
21. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* (2010) **207**:1247–60. doi:10.1084/jem.20092140
22. Poulin LF, Reyal Y, Uronen-Hansson H, Schraml BU, Sancho D, Murphy KM, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. *Blood* (2012) **119**:6052–62. doi:10.1182/blood-2012-01-406967
23. Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, et al. Human tissues contain CD141(hi) cross-presenting dendritic cells with functional homology to mouse CD103(+) nonlymphoid dendritic cells. *Immunity* (2012) **37**:60–73. doi:10.1016/j.immuni.2012.04.012
24. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* (2013) **38**:970–83. doi:10.1016/j.immuni.2013.04.011
25. McGovern N, Schlitzer A, Gunawan M, Jardine L, Shin A, Poyner E, et al. Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages. *Immunity* (2014) **41**:465–77. doi:10.1016/j.immuni.2014.08.006
26. Watchmaker PB, Lahl K, Lee M, Baumjohann D, Morton J, Kim SJ, et al. Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. *Nat Immunol* (2014) **15**:98–108. doi:10.1038/ni.2768
27. Lee J, Breton G, Oliveira TY, Zhou YJ, Aljoufi A, Puhr S, et al. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. *J Exp Med* (2015) **212**:385–99. doi:10.1084/jem.20141442
28. Breton G, Lee J, Zhou YJ, Schreiber JJ, Keler T, Puhr S, et al. Circulating precursors of human CD1c+ and CD141+ dendritic cells. *J Exp Med* (2015) **212**:401–13. doi:10.1084/jem.20141441
29. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature* (2002) **420**:520–62. doi:10.1038/nature01262
30. Haniffa M, Collin M, Ginhoux F. Ontogeny and functional specialization of dendritic cells in human and mouse. *Adv Immunol* (2013) **120**:1–49. doi:10.1016/B978-0-12-417028-5.00001-6
31. Haniffa M, Gunawan M, Jardine L. Human skin dendritic cells in health and disease. *J Dermatol Sci* (2015) **77**(2):85–92. doi:10.1016/j.jdermsci.2014.08.012
32. Collin M, Bigley V, Haniffa M, Hambleton S. Human dendritic cell deficiency: the missing ID? *Nat Rev Immunol* (2011) **11**:575–83. doi:10.1038/nri3046
33. Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* (2003) **19**:59–70. doi:10.1016/S1074-7613(03)00171-7
34. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against *Leishmania*. *Immunity* (2007) **26**:519–31. doi:10.1016/j.immuni.2007.01.017
35. Serbina NV, Hohl TM, Cherny M, Pamer EG. Selective expansion of the monocytic lineage directed by bacterial infection. *J Immunol* (2009) **183**:1900–10. doi:10.4049/jimmunol.0900612
36. de Jong E, Suddason T, Lord GM. Translational mini-review series on Th17 cells: development of mouse and human T helper 17 cells. *Clin Exp Immunol* (2010) **159**:148–58. doi:10.1111/j.1365-2249.2009.04041.x
37. Noster R, Riedel R, Mashreghi MF, Radbruch H, Harms L, Haftmann C, et al. IL-17 and GM-CSF expression are antagonistically regulated by human T helper cells. *Sci Transl Med* (2014) **6**:241ra80. doi:10.1126/scitranslmed.3008706
38. Codarri L, Gyulveszi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol* (2011) **12**:560–7. doi:10.1038/ni.2027
39. Roncarolo MG, Battaglia M. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. *Nat Rev Immunol* (2007) **7**:585–98. doi:10.1038/nri2138
40. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol* (2004) **172**:2731–8. doi:10.4049/jimmunol.172.5.2731
41. Van Rhijn I, Ly D, Moody DB. CD1a, CD1b, and CD1c in immunity against mycobacteria. *Adv Exp Med Biol* (2013) **783**:181–97. doi:10.1007/978-1-4614-6111-1_10
42. van de Mortel JE, Aarts MG. Comparative transcriptomics – model species lead the way. *New Phytol* (2006) **170**:199–201. doi:10.1111/j.1469-8137.2006.01708.x
43. Shay T, Jojic V, Zuk O, Rothamel K, Puyraimond-Zemmour D, Feng T, et al. Conservation and divergence in the transcriptional programs of the human and mouse immune systems. *Proc Natl Acad Sci U S A* (2013) **110**:2946–51. doi:10.1073/pnas.1222738110
44. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* (2005) **102**:15545–50. doi:10.1073/pnas.0506580102
45. Lamb J, Crawford ED, Peck D, Modell JW I, Blat C, Wrobel MJ, et al. The connectivity map: using gene-expression signatures to connect small molecules, genes, and disease. *Science* (2006) **313**:1929–35. doi:10.1126/science.1132939
46. Lindstedt M, Lundberg K, Borrebaeck CA. Gene family clustering identifies functionally associated subsets of human in vivo blood and tonsillar dendritic cells. *J Immunol* (2005) **175**:4839–46. doi:10.4049/jimmunol.175.8.4839
47. Segura E, Touzot M, Bohineust A, Cappuccio A, Chiocchia G, Hosmalin A, et al. Human inflammatory dendritic cells induce th17 cell differentiation. *Immunity* (2013) **38**:336–48. doi:10.1016/j.immuni.2012.10.018
48. Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science* (2014) **343**:776–9. doi:10.1126/science.1247651
49. Mahata B, Zhang X, Kolodziejczyk AA, Proserpio V, Haim-Vilmovsky L, Taylor AE, et al. Single-cell RNA sequencing reveals T helper cells synthesizing steroids de novo to contribute to immune homeostasis. *Cell Rep* (2014) **7**:1130–42. doi:10.1016/j.celrep.2014.04.011
50. Trapnell C, Cacchiarelli D, Grimsby J, Pokharel P, Li S, Morse M, et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat Biotechnol* (2014) **32**:381–6. doi:10.1038/nbt.2859
51. Treutlein B, Brownfield DG, Wu AR, Neff NF, Mantalas GL, Espinoza FH, et al. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. *Nature* (2014) **509**:371–5. doi:10.1038/nature13173
52. Durruthy-Durruthy R, Gottlieb A, Hartman BH, Waldhaus J, Laske RD, Altman R, et al. Reconstruction of the mouse otocyst and early neuroblast lineage at single-cell resolution. *Cell* (2014) **157**:964–78. doi:10.1016/j.cell.2014.03.036
53. Arsenio J, Kakaradov B, Metz PJ, Kim SH, Yeo GW, Chang JT. Early specification of CD8+ T lymphocyte fates during adaptive immunity revealed by single-cell gene-expression analyses. *Nat Immunol* (2014) **15**:365–72. doi:10.1038/ni.2842
54. Luber CA, Cox J, Lauterbach H, Fancke B, Selbach M, Tschopp J, et al. Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity* (2010) **32**:279–89. doi:10.1016/j.immuni.2010.01.013
55. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* (2013) **14**:821–30. doi:10.1038/ni.2638
56. Amir, el- AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol* (2013) **31**:545–52. doi:10.1038/nbt.2594
57. Becher B, Schlitzer A, Chen J, Mair F, Sumatoh HR, Teng KW, et al. High-dimensional analysis of the murine myeloid cell system. *Nat Immunol* (2014) **15**(12):1181–9. doi:10.1038/ni.3006

58. Segura E, Amigorena S. Cross-presentation by human dendritic cell subsets. *Immunol Lett* (2014) **158**:73–8. doi:10.1016/j.imlet.2013.12.001
59. Bigley V, Haniffa M, Doulatov S, Wang XN, Dickinson R, McGovern N, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. *J Exp Med* (2011) **208**:227–34. doi:10.1084/jem.20101459
60. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. IRF8 mutations and human dendritic-cell immunodeficiency. *N Engl J Med* (2011) **365**(2):127–38. doi:10.1056/NEJMoa1100066
61. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**(8):571–8. doi:10.1038/nri3712
62. Naik SH, Sathe P, Park HY, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol* (2007) **8**:1217–26. doi:10.1038/ni1522
63. Onai N, Kurabayashi K, Hosoi-Amaike M, Toyama-Sorimachi N, Matsushima K, Inaba K, et al. A clonogenic progenitor with prominent plasmacytoid dendritic cell developmental potential. *Immunity* (2013) **38**:943–57. doi:10.1016/j.immuni.2013.04.006
64. Galibert L, Diemer GS, Liu Z, Johnson RS, Smith JL, Walzer T, et al. Nectin-like protein 2 defines a subset of T-cell zone dendritic cells and is a ligand for class-I-restricted T-cell-associated molecule. *J Biol Chem* (2005) **280**:21955–64. doi:10.1074/jbc.M502095200
65. Bachem A, Hartung E, Guttler S, Mora A, Zhou X, Hegemann A, et al. Expression of XCR1 characterizes the Batf3-dependent lineage of dendritic cells capable of antigen cross-presentation. *Front Immunol* (2012) **3**:214. doi:10.3389/fimmu.2012.00214
66. Contreras V, Urien C, Guiton R, Alexandre Y, Vu Manh TP, Andrieu T, et al. Existence of CD8alpha-like dendritic cells with a conserved functional specialization and a common molecular signature in distant mammalian species. *J Immunol* (2010) **185**:3313–25. doi:10.4049/jimmunol.1000824
67. Tatsumi K, Taatjes DJ, Wadsworth MP, Bouchard BA, Bovill EG. Cell adhesion molecule 1 (CADM1) is ubiquitously present in the endothelium and smooth muscle cells of the human macro- and micro-vasculature. *Histochem Cell Biol* (2012) **138**:815–20. doi:10.1007/s00418-012-1024-2
68. Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* (2013) **154**:843–58. doi:10.1016/j.cell.2013.07.014
69. Ginhoux F, Collin MP, Bogunovic M, Abel M, Leboeuf M, Helft J, et al. Blood-derived dermal langerin+ dendritic cells survey the skin in the steady state. *J Exp Med* (2007) **204**:3133–46. doi:10.1084/jem.20071733
70. Bigley V, McGovern N, Milne P, Dickinson R, Pagan S, Cookson S, et al. Langerin-expressing dendritic cells in human tissues are related to CD1c+ dendritic cells and distinct from Langerhans cells and CD141high XCR1+ dendritic cells. *J Leukoc Biol* (2014) **97**(4):627–34. doi:10.1189/jlb.1HI0714-351R
71. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen JL, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**:1261–71. doi:10.1084/jem.20092618
72. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* (2008) **322**:1097–100. doi:10.1126/science.1164206
73. Schiavoni G, Mattei F, Sestili P, Borghi P, Venditti M, Morse HCR, et al. ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells. *J Exp Med* (2002) **196**:1415–25. doi:10.1084/jem.20021263
74. Hacker C, Kirsch RD, Ju XS, Hieronymus T, Gust TC, Kuhl C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat Immunol* (2003) **4**:380–6. doi:10.1038/ni903
75. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* (2009) **206**:3115–30. doi:10.1084/jem.20091756
76. Kashiwada M, Pham NL, Pewe LL, Harty JT, Rothman PB. NFIL3/E4BP4 is a key transcription factor for CD8alpha(+) dendritic cell development. *Blood* (2011) **117**:6193–7. doi:10.1182/blood-2010-07-295873
77. Tussiwand R, Lee WL, Murphy TL, Mashayekhi M, KC W, Albring JC, et al. Compensatory dendritic cell development mediated by BATF-IRF interactions. *Nature* (2012) **490**:502–7. doi:10.1038/nature11531
78. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(–) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* (2000) **192**:1685–96. doi:10.1084/jem.192.12.1685
79. Iyoda T, Shimoyama S, Liu K, Omatsu Y, Akiyama Y, Maeda Y, et al. The CD8+ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. *J Exp Med* (2002) **195**:1289–302. doi:10.1084/jem.20020161
80. Bedoui S, Whitney PG, Waithman J, Eidsmo L, Wakim L, Caminschi I, et al. Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. *Nat Immunol* (2009) **10**:488–95. doi:10.1038/ni.1724
81. Hemont C, Neel A, Heslan M, Braudeau C, Josien R. Human blood mDC subsets exhibit distinct TLR repertoire and responsiveness. *J Leukoc Biol* (2013) **93**:599–609. doi:10.1189/jlb.0912452
82. Schulz O, Edwards AD, Schito M, Aliberti J, Manickasingham S, Sher A, et al. CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells in vivo requires a microbial priming signal. *Immunity* (2000) **13**:453–62. doi:10.1016/S1074-7613(00)00045-5
83. Lauterbach H, Bathke B, Gilles S, Traidl-Hoffmann C, Luber CA, Fejer G, et al. Mouse CD8alpha+ DCs and human BDCA3+ DCs are major producers of IFN-lambda in response to poly IC. *J Exp Med* (2010) **207**:2703–17. doi:10.1084/jem.20092720
84. Savina A, Peres A, Cebrian I, Carmo N, Moita C, Hacohen N, et al. The small GTPase Rac2 controls phagosomal alkalinization and antigen crosspresentation selectively in CD8(+) dendritic cells. *Immunity* (2009) **30**:544–55. doi:10.1016/j.immuni.2009.01.013
85. Lin ML, Zhan Y, Proietto AI, Prato S, Wu L, Heath WR, et al. Selective suicide of cross-presenting CD8+ dendritic cells by cytochrome c injection shows functional heterogeneity within this subset. *Proc Natl Acad Sci U S A* (2008) **105**:3029–34. doi:10.1073/pnas.0712394105
86. Desch AN, Gibbings SL, Clambey ET, Janssen WJ, Slansky JE, Kedl RM, et al. Dendritic cell subsets require cis-activation for cytotoxic CD8 T-cell induction. *Nat Commun* (2014) **5**:4674. doi:10.1038/ncomms5674
87. Cohn L, Chatterjee B, Esselborn F, Smed-Sorensen A, Nakamura N, Chalouni C, et al. Antigen delivery to early endosomes eliminates the superiority of human blood BDCA3+ dendritic cells at cross presentation. *J Exp Med* (2013) **210**:1049–63. doi:10.1084/jem.20121251
88. Segura E, Durand M, Amigorena S. Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. *J Exp Med* (2013) **210**:1035–47. doi:10.1084/jem.20121103
89. Nizzoli G, Krietsch J, Weick A, Steinfelder S, Facciotti F, Gruarin P, et al. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* (2013) **122**:932–42. doi:10.1182/blood-2013-04-495424
90. Mashayekhi M, Sandau MM I, Dunay R, Frickel EM, Khan A, Goldszmid RS, et al. CD8alpha(+) dendritic cells are the critical source of interleukin-12 that controls acute infection by *Toxoplasma gondii* tachyzoites. *Immunity* (2011) **35**:249–59. doi:10.1016/j.immuni.2011.08.008
91. Martinez-Lopez M, Iborra S, Conde-Garrosa R, Sancho D. Batf3-dependent CD103+ dendritic cells are major producers of IL-12 that drive local Th1 immunity against *Leishmania* major infection in mice. *Eur J Immunol* (2015) **45**:119–29. doi:10.1002/eji.201444651
92. Nakano H, Free ME, Whitehead GS, Maruoka S, Wilson RH, Nakano K, et al. Pulmonary CD103(+) dendritic cells prime Th2 responses to inhaled allergens. *Mucosal Immunol* (2012) **5**:53–65. doi:10.1038/mi.2011.47
93. Segura E, Valladeau-Guilemond J, Donnadieu M-H, Sastre-Garau X, Soumelis V, Amigorena S. Characterization of resident and migratory dendritic cells in human lymph nodes. *J Exp Med* (2012) **209**:653–60. doi:10.1084/jem.20111457
94. Yu CI, Becker C, Metang P, Marches F, Wang Y, Toshiyuki H, et al. Human CD141+ dendritic cells induce CD4+ T cells to produce type 2 cytokines. *J Immunol* (2014) **193**(9):4335–43. doi:10.4049/jimmunol.1401159
95. Ginhoux F, Schlitzer A. CD11b+ DCs rediscovered: implications for vaccination. *Expert Rev Vaccines* (2014) **13**:445–7. doi:10.1586/14760584.2014.893196
96. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. *Immunity* (2011) **35**:780–91. doi:10.1016/j.immuni.2011.08.013
97. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* (2013) **39**:925–38. doi:10.1016/j.immuni.2013.10.004

98. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* (2013) **38**:322–35. doi:10.1016/j.immuni.2012.10.016
99. Wollenberg A, Mommaas M, Oppel T, Schottdorf EM, Gunther S, Moderer M. Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. *J Invest Dermatol* (2002) **118**:327–34. doi:10.1046/j.0022-202x.2001.01665.x
100. Zaba LC, Fuentes-Duculan J, Steinman RM, Krueger JG, Lowes MA. Normal human dermis contains distinct populations of CD11c+BDCA-1+ dendritic cells and CD163+FXIIIA+ macrophages. *J Clin Invest* (2007) **117**:2517–25. doi:10.1172/JCI32282
101. Ochoa MT, Loncaric A, Krutzik SR, Becker TC, Modlin RL. Dermal dendritic cells" comprise two distinct populations: CD1(+) dendritic cells and CD209(+) macrophages. *J Invest Dermatol* (2008) **128**(9):2225–31. doi:10.1038/jid.2008.56
102. Wu L, D'Amico A, Winkel KD, Suter M, Lo D, Shortman K. RelB is essential for the development of myeloid-related CD8alpha- dendritic cells but not of lymphoid-related CD8alpha+ dendritic cells. *Immunity* (1998) **9**:839–47. doi:10.1016/S1074-7613(00)80649-4
103. Guerriero A, Langmuir PB, Spain LM, Scott EW. PU.1 is required for myeloid-derived but not lymphoid-derived dendritic cells. *Blood* (2000) **95**:879–85.
104. Suzuki S, Honma K, Matsuyama T, Suzuki K, Toriyama K, Akitoyo I, et al. Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha- dendritic cell development. *Proc Natl Acad Sci U S A* (2004) **101**:8981–6. doi:10.1073/pnas.0402139101
105. Tamura T, Tailor P, Yamaoka K, Kong HJ, Tsujimura H, O'Shea JJ, et al. IFN regulatory factor-4 and -8 govern dendritic cell subset development and their functional diversity. *J Immunol* (2005) **174**:2573–81. doi:10.4049/jimmunol.174.5.2573
106. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J Exp Med* (2007) **204**:1653–64.
107. Satpathy AT, Briseno CG, Lee JS, Ng D, Manieri NA, Kc W, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol* (2013) **14**:937–48. doi:10.1038/ni.2679
108. Williams JW, Tjota MY, Clay BS, Vander Lugt B, Bandukwala HS, Hrusch CL, et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat Commun* (2013) **4**:2990. doi:10.1038/ncomms3990
109. Vander Lugt B, Khan AA, Hackney JA, Agrawal S, Lesch J, Zhou M, et al. Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. *Nat Immunol* (2014) **15**:161–7. doi:10.1038/ni.2795
110. Brass AL, Kehrli E, Eisenbeis CF, Storb U, Singh H. Pip, a lymphoid-restricted IRF, contains a regulatory domain that is important for autoinhibition and ternary complex formation with the Ets factor PU.1. *Genes Dev* (1996) **10**:2335–47. doi:10.1101/gad.10.18.2335
111. Carotta S, Dakic A, D'Amico A, Pang SH, Greig KT, Nutt SL, et al. The transcription factor PU.1 controls dendritic cell development and Flt3 cytokine receptor expression in a dose-dependent manner. *Immunity* (2010) **32**:628–41. doi:10.1016/j.immuni.2010.05.005
112. Maraskovsky E, Daro E, Roux E, Teepe M, Maliszewski CR, Hoek J, et al. In vivo generation of human dendritic cell subsets by Flt3 ligand. *Blood* (2000) **96**:878–84.
113. Dakic A, Wu L, Nutt SL. Is PU.1 a dosage-sensitive regulator of haemopoietic lineage commitment and leukaemogenesis? *Trends Immunol* (2007) **28**:108–14. doi:10.1016/j.it.2007.01.006
114. Tailor P, Tamura T, Morse HCR, Ozato K. The BXH2 mutation in IRF8 differentially impairs dendritic cell subset development in the mouse. *Blood* (2008) **111**:1942–5. doi:10.1182/blood-2007-07-100750
115. Kurotaki D, Osato N, Nishiyama A, Yamamoto M, Ban T, Sato H, et al. Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation. *Blood* (2013) **121**:1839–49. doi:10.1182/blood-2012-06-437863
116. Yanez A, Ng MY, Hassanzadeh-Kiabi N, Goodridge HS. IRF8 acts in lineage-committed rather than oligopotent progenitors to control neutrophil vs monocyte production. *Blood* (2015) **125**:1452–9. doi:10.1182/blood-2014-09-600833
117. Dudziak D, Kamphorst AO, Heidkamp GF, Buchholz VR, Trumpfheller C, Yamazaki S, et al. Differential antigen processing by dendritic cell subsets in vivo. *Science* (2007) **315**:107–11. doi:10.1126/science.1136080
118. Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hagerbrand K, Marsal J, et al. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* (2013) **38**:958–69. doi:10.1016/j.immuni.2013.03.009
119. Gao Y, Nish SA, Jiang R, Hou L, Licona-Limon P, Weinstein JS, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* (2013) **39**:722–32. doi:10.1016/j.immuni.2013.08.028
120. Zhou Q, Ho AW, Schlitzer A, Tang Y, Wong KH, Wong FH, et al. GM-CSF-licensed CD11b+ lung dendritic cells orchestrate Th2 immunity to blomia tropicalis. *J Immunol* (2014) **193**:496–509. doi:10.4049/jimmunol.1303138
121. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* (2004) **5**:987–95. doi:10.1038/ni1112
122. Moret FM, Hack CE, van der Wurff-Jacobs KM, de Jager W, Radstake TR, Lafeber FP, et al. Intra-articular CD1c-expressing myeloid dendritic cells from rheumatoid arthritis patients express a unique set of T cell-attracting chemokines and spontaneously induce Th1, Th17 and Th2 cell activity. *Arthritis Res Ther* (2013) **15**:R155. doi:10.1186/ar4338
123. Kassianos AJ, Wang X, Sampangi S, Muczynski K, Healy H, Wilkinson R. Increased tubulointerstitial recruitment of human CD141(hi) CLEC9A(+) and CD1c(+) myeloid dendritic cell subsets in renal fibrosis and chronic kidney disease. *Am J Physiol Renal Physiol* (2013) **305**:F1391–401. doi:10.1152/ajprenal.00318.2013
124. Jahnsen FL, Moloney ED, Hogan T, Upham JW, Burke CM, Holt PG. Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge. *Thorax* (2001) **56**:823–6. doi:10.1136/thorax.56.11.823
125. Yu CI, Becker C, Wang Y, Marches F, Helft J, Leboeuf M, et al. Human CD1c dendritic cells drive the differentiation of CD103 CD8 mucosal effector T cells via the cytokine TGF-beta. *Immunity* (2013) **38**(4):818–30. doi:10.1016/j.immuni.2013.03.004
126. Klechevsky E, Morita R, Liu M, Cao Y, Coquery S, Thompson-Snipes L, et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity* (2008) **29**:497–510. doi:10.1016/j.immuni.2008.07.013
127. Haniffa M, Ginhoux F, Wang XN, Bigley V, Abel M, Dimmick I, et al. Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. *J Exp Med* (2009) **206**:371–85. doi:10.1084/jem.20081633
128. O'Doherty U, Peng M, Gezelter S, Swiggard WJ, Betjes M, Bhardwaj N, et al. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology* (1994) **82**:487–93.
129. Grouard G, Rissoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med* (1997) **185**:1101–11. doi:10.1084/jem.185.6.1101
130. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* (1999) **5**:919–23. doi:10.1038/11360
131. Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science* (1999) **284**:1835–7. doi:10.1126/science.284.5421.1835
132. Satpathy AT, Wu X, Albring JC, Murphy KM. Re(de)fining the dendritic cell lineage. *Nat Immunol* (2012) **13**:1145–54. doi:10.1038/ni.2467
133. Taussig DC, Pearce DJ, Simpson C, Rohatiner AZ, Lister TA, Kelly G, et al. Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. *Blood* (2005) **106**:4086–92. doi:10.1182/blood-2005-03-1072
134. Toba K, Koike T, Shibata A, Hashimoto S, Takahashi M, Masuko M, et al. Novel technique for the direct flow cytofluorometric analysis of human basophils in unseparated blood and bone marrow, and the characterization of phenotype and peroxidase of human basophils. *Cytometry* (1999) **35**:249–59. doi:10.1002/(SICI)1097-0320(19990301)35:3<249::AID-CYTO8>3.3.CO;2-F
135. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* (2000) **165**:6037–46. doi:10.4049/jimmunol.165.11.6037
136. Dzionek A, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med* (2001) **194**:1823–34. doi:10.1084/jem.194.12.1823
137. Lebre MC, Jongbloed SL, Tas SW, Smeets TJ I, McInnes B, Tak PP. Rheumatoid arthritis synovium contains two subsets of CD83-DC-LAMP- dendritic cells

with distinct cytokine profiles. *Am J Pathol* (2008) **172**:940–50. doi:10.2353/ajpath.2008.070703
138. Schlitzer A, Loschko J, Mair K, Vogelmann R, Henkel L, Einwachter H, et al. Identification of CCR9- murine plasmacytoid DC precursors with plasticity to differentiate into conventional DCs. *Blood* (2011) **117**:6562–70. doi:10.1182/blood-2010-12-326678
139. Schlitzer A, Heiseke AF, Einwachter H, Reindl W, Schiemann M, Manta CP, et al. Tissue-specific differentiation of a circulating CCR9- pDC-like common dendritic cell precursor. *Blood* (2012) **119**:6063–71. doi:10.1182/blood-2012-03-418400
140. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* (2008) **135**:37–48. doi:10.1016/j.cell.2008.09.016
141. Ghosh HS, Cisse B, Bunin A, Lewis KL, Reizis B. Continuous expression of the transcription factor e2-2 maintains the cell fate of mature plasmacytoid dendritic cells. *Immunity* (2010) **33**:905–16. doi:10.1016/j.immuni.2010.11.023
142. Peippo M, Ignatius J. Pitt-Hopkins syndrome. *Mol Syndromol* (2012) **2**:171–80.
143. Sasaki I, Hoshino K, Sugiyama T, Yamazaki C, Yano T, Iizuka A, et al. Spi-B is critical for plasmacytoid dendritic cell function and development. *Blood* (2012) **120**:4733–43. doi:10.1182/blood-2012-06-436527
144. Schotte R, Nagasawa M, Weijer K, Spits H, Blom B. The ETS transcription factor Spi-B is required for human plasmacytoid dendritic cell development. *J Exp Med* (2004) **200**:1503–9. doi:10.1084/jem.20041231
145. Hochrein H, O'Keeffe M, Wagner H. Human and mouse plasmacytoid dendritic cells. *Hum Immunol* (2002) **63**:1103–10. doi:10.1016/S0198-8859(02)00748-6
146. Fuchsberger M, Hochrein H, O'Keeffe M. Activation of plasmacytoid dendritic cells. *Immunol Cell Biol* (2005) **83**:571–7. doi:10.1111/j.1440-1711.2005.01392.x
147. Jarrossay D, Napolitani G, Colonna M, Sallusto F, Lanzavecchia A. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol* (2001) **31**:3388–93. doi:10.1002/1521-4141(200111)31:11<3388::AID-IMMU3388>3.0.CO;2-Q
148. Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* (2001) **194**:863–9. doi:10.1084/jem.194.6.863
149. Demaria O, Pagni PP, Traub S, de Gassart A, Branzk N, Murphy AJ, et al. TLR8 deficiency leads to autoimmunity in mice. *J Clin Invest* (2010) **120**:3651–62. doi:10.1172/JCI42081
150. Desnues B, Macedo AB, Roussel-Queval A, Bonnardel J, Henri S, Demaria O, et al. TLR8 on dendritic cells and TLR9 on B cells restrain TLR7-mediated spontaneous autoimmunity in C57BL/6 mice. *Proc Natl Acad Sci U S A* (2014) **111**:1497–502. doi:10.1073/pnas.1314121111
151. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol* (2008) **8**:594–606. doi:10.1038/nri2358
152. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci Transl Med* (2011) **3**:73ra20. doi:10.1126/scitranslmed.3001201
153. Murdoch C, Tazzyman S, Webster S, Lewis CE. Expression of tie-2 by human monocytes and their responses to angiopoietin-2. *J Immunol* (2007) **178**:7405–11. doi:10.4049/jimmunol.178.11.7405
154. Schakel K, Kannagi R, Kniep B, Goto Y, Mitsuoka C, Zwirner J, et al. 6-Sulfo LacNAc, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human dendritic cells. *Immunity* (2002) **17**:289–301. doi:10.1016/S1074-7613(02)00393-X
155. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* (2010) **33**:375–86. doi:10.1016/j.immuni.2010.08.012
156. Rosenbauer F, Tenen DG. Transcription factors in myeloid development: balancing differentiation with transformation. *Nat Rev Immunol* (2007) **7**:105–17. doi:10.1038/nri2024
157. Staber PB, Zhang P, Ye M, Welner RS, Nombela-Arrieta C, Bach C, et al. Sustained PU.1 levels balance cell-cycle regulators to prevent exhaustion of adult hematopoietic stem cells. *Mol Cell* (2013) **49**:934–46. doi:10.1016/j.molcel.2013.01.007
158. Scott EW, Simon MC, Anastasi J, Singh H. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. *Science* (1994) **265**:1573–7. doi:10.1126/science.8079170
159. Kueh HY, Champhekar A, Nutt SL, Elowitz MB, Rothenberg EV. Positive feedback between PU.1 and the cell cycle controls myeloid differentiation. *Science* (2013) **341**:670–3. doi:10.1126/science.1240831
160. Laslo P, Spooner CJ, Warmflash A, Lancki DW, Lee HJ, Sciammas R, et al. Multilineage transcriptional priming and determination of alternate hematopoietic cell fates. *Cell* (2006) **126**:755–66. doi:10.1016/j.cell.2006.06.052
161. Rosa A, Ballarino M, Sorrentino A, Sthandier O, De Angelis FG, Marchioni M, et al. The interplay between the master transcription factor PU.1 and miR-424 regulates human monocyte/macrophage differentiation. *Proc Natl Acad Sci U S A* (2007) **104**:19849–54. doi:10.1073/pnas.0706963104
162. Kurotaki D, Yamamoto M, Nishiyama A, Uno K, Ban T, Ichino M, et al. IRF8 inhibits C/EBPalpha activity to restrain mononuclear phagocyte progenitors from differentiating into neutrophils. *Nat Commun* (2014) **5**:4978. doi:10.1038/ncomms5978
163. Hanna RN, Carlin LM, Hubbeling HG, Nackiewicz D, Green AM, Punt JA, et al. The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C- monocytes. *Nat Immunol* (2011) **12**:778–85. doi:10.1038/ni.2063
164. Bakri Y, Sarrazin S, Mayer UP, Tillmanns S, Nerlov C, Boned A, et al. Balance of MafB and PU.1 specifies alternative macrophage or dendritic cell fate. *Blood* (2005) **105**:2707–16. doi:10.1182/blood-2004-04-1448
165. Lehtonen A, Veckman V, Nikula T, Lahesmaa R, Kinnunen L, Matikainen S, et al. Differential expression of IFN regulatory factor 4 gene in human monocyte-derived dendritic cells and macrophages. *J Immunol* (2005) **175**:6570–9. doi:10.4049/jimmunol.175.10.6570
166. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* (2011) **12**:231–8. doi:10.1038/ni.1990
167. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* (2010) **11**:936–44. doi:10.1038/ni.1920
168. Derlindati E, Dei Cas A, Montanini B, Spigoni V, Curella V, Aldigeri R, et al. Transcriptomic analysis of human polarized macrophages: more than one role of alternative activation? *PLoS One* (2015) **10**:e0119751. doi:10.1371/journal.pone.0119751
169. Martinez FO, Helming L, Milde R, Varin A, Melgert BN, Draijer C, et al. Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: similarities and differences. *Blood* (2013) **121**:e57–69. doi:10.1182/blood-2012-06-436212
170. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) **15**:929–37. doi:10.1038/ni.2967
171. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. *Mucosal Immunol* (2013) **6**:498–510. doi:10.1038/mi.2012.89
172. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* (2007) **204**:171–80. doi:10.1084/jem.20061011
173. Cheong C, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP, et al. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* (2010) **143**:416–29. doi:10.1016/j.cell.2010.09.039
174. Nakano H, Lin KL, Yanagita M, Charbonneau C, Cook DN, Kakiuchi T, et al. Blood-derived inflammatory dendritic cells in lymph nodes stimulate acute T helper type 1 immune responses. *Nat Immunol* (2009) **10**:394–402. doi:10.1038/ni.1707
175. Bosschaerts T, Guilliams M, Stijlemans B, Morias Y, Engel D, Tacke F, et al. Tip-DC development during parasitic infection is regulated by IL-10 and requires CCL2/CCR2, IFN-gamma and MyD88 signaling. *PLoS Pathog* (2010) **6**:e1001045. doi:10.1371/journal.ppat.1001045
176. Hohl TM, Rivera A, Lipuma L, Gallegos A, Shi C, Mack M, et al. Inflammatory monocytes facilitate adaptive CD4 T cell responses during respiratory fungal infection. *Cell Host Microbe* (2009) **6**:470–81. doi:10.1016/j.chom.2009.10.007
177. Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, et al. Minimal differentiation of classical monocytes as they survey steady-state

tissues and transport antigen to lymph nodes. *Immunity* (2013) **39**:599–610. doi:10.1016/j.immuni.2013.08.007
178. Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. *Immunol Res* (2012) **53**:41–57. doi:10.1007/s12026-012-8297-3
179. Hansel A, Gunther C, Ingwersen J, Starke J, Schmitz M, Bachmann M, et al. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong TH17/TH1 T-cell responses. *J Allergy Clin Immunol* (2011) **127**:.e1–9. doi:10.1016/j.jaci.2010.12.009
180. Brumeanu TD, Swiggard WJ, Steinman RM, Bona CA, Zaghouani H. Efficient loading of identical viral peptide onto class II molecules by antigenized immunoglobulin and influenza virus. *J Exp Med* (1993) **178**:1795–9. doi:10.1084/jem.178.5.1795
181. Matthews K, Chung NP, Klasse PJ, Moore JP, Sanders RW. Potent induction of antibody-secreting B cells by human dermal-derived CD14+ dendritic cells triggered by dual TLR ligation. *J Immunol* (2012) **189**:5729–44. doi:10.4049/jimmunol.1200601
182. Birbeck MS, Breathnach AS, Everall JD. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. *J Invest Dermatol* (1961) **37**:51–64. doi:10.1038/jid.1961.7
183. Kissenpfennig A, Ait-Yahia S, Clair-Moninot V, Stossel H, Badell E, Bordat Y, et al. Disruption of the langerin/CD207 gene abolishes birbeck granules without a marked loss of Langerhans cell function. *Mol Cell Biol* (2005) **25**:88–99. doi:10.1128/MCB.25.1.88-99.2005
184. Kubo A, Nagao K, Yokouchi M, Sasaki H, Amagai M. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. *J Exp Med* (2009) **206**:2937–46. doi:10.1084/jem.20091527
185. Teunissen MB, Haniffa M, Collin MP. Insight into the immunobiology of human skin and functional specialization of skin dendritic cell subsets to innovate intradermal vaccination design. *Curr Top Microbiol Immunol* (2012) **351**:25–76. doi:10.1007/82_2011_169
186. Sere K, Baek JH, Ober-Blobaum J, Muller-Newen G, Tacke F, Yokota Y, et al. Two distinct types of Langerhans cells populate the skin during steady state and inflammation. *Immunity* (2012) **37**:905–16. doi:10.1016/j.immuni.2012.07.019
187. Nagao K, Kobayashi T, Moro K, Ohyama M, Adachi T, Kitashima DY, et al. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. *Nat Immunol* (2012) **13**:744–52. doi:10.1038/ni.2353
188. Chopin M, Seillet C, Chevrier S, Wu L, Wang H, Morse HCR, et al. Langerhans cells are generated by two distinct PU.1-dependent transcriptional networks. *J Exp Med* (2013) **210**:2967–80. doi:10.1084/jem.20130930
189. Chorro L, Sarde A, Li M, Woollard KJ, Chambon P, Malissen B, et al. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. *J Exp Med* (2009) **206**:3089–100. doi:10.1084/jem.20091586
190. Kanitakis J, Petruzzo P, Dubernard JM. Turnover of epidermal Langerhans' cells. *N Engl J Med* (2004) **351**:2661–2. doi:10.1056/NEJM200412163512523
191. Kanitakis J, Morelon E, Petruzzo P, Badet L, Dubernard JM. Self-renewal capacity of human epidermal Langerhans cells: observations made on a composite tissue allograft. *Exp Dermatol* (2011) **20**:145–6. doi:10.1111/j.1600-0625.2010.01146.x
192. Martinez-Cingolani C, Grandclaudon M, Jeanmougin M, Jouve M, Zollinger R, Soumelis V. Human blood BDCA-1 dendritic cells differentiate into Langerhans-like cells with thymic stromal lymphopoietin and TGF-beta. *Blood* (2014) **124**:2411–20. doi:10.1182/blood-2014-04-568311
193. Milne P, Bigley V, Gunawan M, Haniffa M, Collin M. CD1c+ blood dendritic cells have Langerhans cell potential. *Blood* (2014) **125**(3):470–3. doi:10.1182/blood-2014-08-593582
194. Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, et al. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol* (2002) **3**:1135–41. doi:10.1038/ni852
195. Collin MP, Hart DN, Jackson GH, Cook G, Cavet J, Mackinnon S, et al. The fate of human Langerhans cells in hematopoietic stem cell transplantation. *J Exp Med* (2006) **203**:27–33. doi:10.1084/jem.20051787
196. Mielcarek M, Kirkorian AY, Hackman RC, Price J, Storer BE, Wood BL, et al. Langerhans cell homeostasis and turnover after nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation. *Transplantation* (2014) **98**(5):563–8. doi:10.1097/TP.0000000000000097
197. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-derived interleukin-34 controls the development and maintenance of Langerhans cells and the maintenance of microglia. *Immunity* (2012) **37**:1050–60. doi:10.1016/j.immuni.2012.11.001
198. Kashihara M, Ueda M, Horiguchi Y, Furukawa F, Hanaoka M, Imamura S. A monoclonal antibody specifically reactive to human Langerhans cells. *J Invest Dermatol* (1986) **87**:602–7. doi:10.1111/1523-1747.ep12455849
199. Stoitzner P, Romani N, McLellan AD, Tripp CH, Ebner S. Isolation of skin dendritic cells from mouse and man. *Methods Mol Biol* (2010) **595**:235–48. doi:10.1007/978-1-60761-421-0_16
200. Fainaru O, Woolf E, Lotem J, Yarmus M, Brenner O, Goldenberg D, et al. Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation. *EMBO J* (2004) **23**:969–79. doi:10.1038/sj.emboj.7600085
201. Chopin M, Nutt SL. Establishing and maintaining the Langerhans cell network. *Semin Cell Dev Biol* (2014). doi:10.1016/j.semcdb.2014.02.001
202. Kaplan DH, Kissenpfennig A, Clausen BE. Insights into Langerhans cell function from Langerhans cell ablation models. *Eur J Immunol* (2008) **38**:2369–76. doi:10.1002/eji.200838397
203. Bennett CL, Fallah-Arani F, Conlan T, Trouillet C, Goold H, Chorro L, et al. Langerhans cells regulate cutaneous injury by licensing CD8 effector cells recruited to the skin. *Blood* (2011) **117**(26):7063–9. doi:10.1182/blood-2011-01-329185
204. Henri S, Poulin LF, Tamoutounour S, Ardouin L, Guilliams M, de Bovis B, et al. CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. *J Exp Med* (2010) **207**:189–206. doi:10.1084/jem.20091964
205. Stoitzner P, Tripp CH, Eberhart A, Price KM, Jung JY, Bursch L, et al. Langerhans cells cross-present antigen derived from skin. *Proc Natl Acad Sci U S A* (2006) **103**:7783–8. doi:10.1073/pnas.0509307103
206. Kashem SW, Igyarto BZ, Gerami-Nejad M, Kumamoto Y, Mohammed J, Jarrett E, et al. Candida albicans morphology and dendritic cell subsets determine T helper cell differentiation. *Immunity* (2015) **42**:356–66. doi:10.1016/j.immuni.2015.01.008
207. Puel A, Cypowyj S, Marodi L, Abel L, Picard C, Casanova JL. Inborn errors of human IL-17 immunity underlie chronic mucocutaneous candidiasis. *Curr Opin Allergy Clin Immunol* (2012) **12**:616–22. doi:10.1097/ACI.0b013e328358cc0b
208. Ni L, Gayet I, Zurawski S, Duluc D, Flamar AL, Li XH, et al. Concomitant activation and antigen uptake via human dectin-1 results in potent antigen-specific CD8+ T cell responses. *J Immunol* (2010) **185**:3504–13. doi:10.4049/jimmunol.1000999
209. Furio L, Briotet I, Journeaux A, Billard H, Peguet-Navarro J. Human Langerhans cells are more efficient than CD14(-)CD1c(+) dermal dendritic cells at priming naive CD4(+) T cells. *J Invest Dermatol* (2010) **130**:1345–54. doi:10.1038/jid.2009.424
210. Mathers AR, Janelsins BM, Rubin JP, Tkacheva OA, Shufesky WJ, Watkins SC, et al. Differential capability of human cutaneous dendritic cell subsets to initiate Th17 responses. *J Immunol* (2009) **182**:921–33. doi:10.4049/jimmunol.182.2.921
211. Fujita H, Nograles KE, Kikuchi T, Gonzalez J, Carucci JA, Krueger JG. Human Langerhans cells induce distinct IL-22-producing CD4+ T cells lacking IL-17 production. *Proc Natl Acad Sci U S A* (2009) **106**:21795–800. doi:10.1073/pnas.0911472106
212. Seneschal J, Clark RA, Gehad A, Baecher-Allan CM, Kupper TS. Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory t cells. *Immunity* (2012) **36**(5):873–84. doi:10.1016/j.immuni.2012.03.018
213. Muller PA, Koscso B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* (2014) **158**:300–13. doi:10.1016/j.cell.2014.04.050
214. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science* (2011) **333**:1456–8. doi:10.1126/science.1202529
215. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature* (2013) **496**:445–55. doi:10.1038/nature12034
216. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* (2014) **40**:91–104. doi:10.1016/j.immuni.2013.11.019

217. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* (2013) **210**:1977–92. doi:10.1084/jem.20131199
218. Sieweke MH, Allen JE. Beyond stem cells: self-renewal of differentiated macrophages. *Science* (2013) **342**:1242974. doi:10.1126/science.1242974
219. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**:1118–28. doi:10.1038/ni.2419
220. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**:1312–26. doi:10.1016/j.cell.2014.11.018
221. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**:1327–40. doi:10.1016/j.cell.2014.11.023
222. Hamann J, Koning N, Pouwels W, Ulfman LH, van Eijk M, Stacey M, et al. EMR1, the human homolog of F4/80, is an eosinophil-specific receptor. *Eur J Immunol* (2007) **37**:2797–802. doi:10.1002/eji.200737553
223. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* (2011) **11**:723–37. doi:10.1038/nri3073
224. Gordon S, Pluddemann A, Martinez Estrada F. Macrophage heterogeneity in tissues: phenotypic diversity and functions. *Immunol Rev* (2014) **262**:36–55. doi:10.1111/imr.12223
225. Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat Neurosci* (2013) **16**:273–80. doi:10.1038/nn.3318
226. Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev Cell* (2002) **3**:889–901. doi:10.1016/S1534-5807(02)00369-6
227. Kohyama M, Ise W, Edelson BT, Wilker PR, Hildner K, Mejia C, et al. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. *Nature* (2009) **457**:318–21. doi:10.1038/nature07472
228. Haldar M, Kohyama M, So AY, Kc W, Wu X, Briseno CG, et al. Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. *Cell* (2014) **156**:1223–34. doi:10.1016/j.cell.2014.01.069
229. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* (2014) **40**:274–88. doi:10.1016/j.immuni.2014.01.006
230. Schroder K, Irvine KM, Taylor MS, Bokil NJ, Le Cao KA, Masterman KA, et al. Conservation and divergence in toll-like receptor 4-regulated gene expression in primary human versus mouse macrophages. *Proc Natl Acad Sci U S A* (2012) **109**:E944–53. doi:10.1073/pnas.1110156109
231. Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci* (2011) **14**:1142–9. doi:10.1038/nn.2887
232. Segura E, Amigorena S. Inflammatory dendritic cells in mice and humans. *Trends Immunol* (2013) **34**:440–5. doi:10.1016/j.it.2013.06.001
233. Zhan Y, Xu Y, Seah S, Brady JL, Carrington EM, Cheers C, et al. Resident and monocyte-derived dendritic cells become dominant IL-12 producers under different conditions and signaling pathways. *J Immunol* (2010) **185**:2125–33. doi:10.4049/jimmunol.0903793
234. Fei M, Bhatia S, Oriss TB, Yarlagadda M, Khare A, Akira S, et al. TNF-alpha from inflammatory dendritic cells (DCs) regulates lung IL-17A/IL-5 levels and neutrophilia versus eosinophilia during persistent fungal infection. *Proc Natl Acad Sci U S A* (2011) **108**:5360–5. doi:10.1073/pnas.1015476108
235. Ikarashi M, Nakashima H, Kinoshita M, Sato A, Nakashima M, Miyazaki H, et al. Distinct development and functions of resident and recruited liver Kupffer cells/macrophages. *J Leukoc Biol* (2013) **94**:1325–36. doi:10.1189/jlb.0313144
236. Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin SL, Nussbaum R, et al. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* (2005) **102**:19057–62. doi:10.1073/pnas.0509736102
237. Fujita H, Shemer A, Suarez-Farinas M, Johnson-Huang LM, Tintle S, Cardinale I, et al. Lesional dendritic cells in patients with chronic atopic dermatitis and psoriasis exhibit parallel ability to activate T-cell subsets. *J Allergy Clin Immunol* (2011) **128**:e1–12. doi:10.1016/j.jaci.2011.05.016
238. Reynolds G, Gibbon JR, Pratt AG, Wood MJ, Coady D, Raftery G, et al. Synovial CD4+ T-cell-derived GM-CSF supports the differentiation of an inflammatory dendritic cell population in rheumatoid arthritis. *Ann Rheum Dis* (2015). doi:10.1136/annrheumdis-2014-206578

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***Correspondence:**
Thien-Phong Vu Manh and Marc Dalod,
Centre d'Immunologie de Marseille-Luminy, Parc Scientifique et Technologique de Luminy, Case 906, Marseille Cedex 9 F-13288, France
vumanh@ciml.univ-mrs.fr;
dalod@ciml.univ-mr.fr
Isabelle Schwartz-Cornil,
UR892, Virologie et Immunologie Moléculaires, INRA, Domaine de Vilvert, Jouy-en-Josas Cedex 78352, France
isabelle.schwartz@jouy.inra.fr

†Present address:
Jamila Elhmouzi-Younes, CEA, Division of Immuno-Virology, IDMIT Center, Institute for Emerging Diseases and Innovative Therapies (iMETI), DSV, Fontenay-aux-Roses, France

‡Thien-Phong Vu Manh and Jamila Elhmouzi-Younes have contributed equally to this work.

§Senior co-authorship

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Defining mononuclear phagocyte subset homology across several distant warm-blooded vertebrates through comparative transcriptomics

Thien-Phong Vu Manh[1,2,3]‡, Jamila Elhmouzi-Younes[4†‡], Céline Urien[4], Suzana Ruscanu[4], Luc Jouneau[4], Mickaël Bourge[5], Marco Moroldo[6], Gilles Foucras[7,8], Henri Salmon[9,10], Hélène Marty[9,10], Pascale Quéré[9,10], Nicolas Bertho[4], Pierre Boudinot[4], Marc Dalod[1,2,3*§] and Isabelle Schwartz-Cornil[4*§]*

[1] UM2, Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, Marseille, France, [2] U1104, INSERM, Marseille, France, [3] UMR7280, CNRS, Marseille, France, [4] UR892, Virologie et Immunologie Moléculaires, INRA, Domaine de Vilvert, Jouy-en-Josas, France, [5] IFR87 La Plante et son Environnement, IMAGIF CNRS, Gif-sur-Yvette, France, [6] CRB GADIE, Génétique Animale et Biologie Intégrative, INRA, Domaine de Vilvert, Jouy-en-Josas, France, [7] UMR1225, Université de Toulouse, INPT, ENVT, Toulouse, France, [8] UMR1225, Interactions Hôtes-Agents Pathogènes, INRA, Toulouse, France, [9] UMR1282, Infectiologie et Santé Publique, INRA, Nouzilly, France, [10] UMR1282, Université François Rabelais de Tours, Tours, France

Mononuclear phagocytes are organized in a complex system of ontogenetically and functionally distinct subsets, that has been best described in mouse and to some extent in human. Identification of homologous mononuclear phagocyte subsets in other vertebrate species of biomedical, economic, and environmental interest is needed to improve our knowledge in physiologic and physio-pathologic processes, and to design intervention strategies against a variety of diseases, including zoonotic infections. We developed a streamlined approach combining refined cell sorting and integrated comparative transcriptomics analyses which revealed conservation of the mononuclear phagocyte organization across human, mouse, sheep, pigs and, in some respect, chicken. This strategy should help democratizing the use of omics analyses for the identification and study of cell types across tissues and species. Moreover, we identified conserved gene signatures that enable robust identification and universal definition of these cell types. We identified new evolutionarily conserved gene candidates and gene interaction networks for the molecular regulation of the development or functions of these cell types, as well as conserved surface candidates for refined subset phenotyping throughout species. A phylogenetic analysis revealed that orthologous genes of the conserved signatures exist in teleost fishes and apparently not in Lamprey.

Keywords: comparative biology, immunology, dendritic cells, monocytes, macrophages, genomic and bio-informatic methods

Introduction

Reaching the global health objective requires to improve disease prevention and treatments in humans and in a wide variety of animal species. To achieve that goal, knowledge of the immune system, and particularly of the mononuclear phagocyte system that orchestrates the immune response, needs to be translated across species in order to develop better vaccines and immune response-targeting therapies in relevant species.

The mononuclear phagocytes encompass three main functional cell types: monocytes (Mo), macrophages (MP), and DC. The main functions of Mo are to patrol the body to detect infections and to produce microbicidal compounds including TNF, superoxide, or nitric oxide intermediates, or to differentiate into MP. The main function of MP is to preserve tissue homeostasis through trophic and scavenger functions. DCs are professional antigen-presenting cells that are key instructors of immunity, controlling tolerance to self and immune defense against pathogens. However, beyond these generic definitions, each of these mononuclear phagocyte category encompasses a complex array of different subtypes with distinct ontogeny and functions, as described extensively in mice and to some extent in humans. Mo include at least two main subsets, classical Mo (cMo) and non-classical Mo (ncMo) (1), that express different innate immune recognition receptors and mediate distinct functions, with ncMo showing the original property of patrolling blood vessels (2). Adult MP are derived either from embryonic precursors and self-renew in tissues, or in some cases are replenished from circulating Mo (2–6). The MP subtypes populating different tissues show distinct molecular and functional characteristics which are in a large part determined by their anatomical microenvironment (7, 8). Two cell types with morphologic and functional features of DC derive from the Mo/MP lineage, namely monocyte-derived DC (MoDC) and Langerhans cells (9). MoDC are generated (i) upon inflammatory stimuli *in vivo* (10), (ii) at steady-state in the skin (3), and (iii) upon culture of purified Mo or of total bone marrow cells with GM-CSF ± IL-4 *in vitro* (11, 12). Langerhans cells derive from embryonic monocytic precursors upon IL-34 signaling and populate the outer layer of epithelia (13). Finally, three types of *bona fide* DC exist, the plasmacytoid DC (pDC) and the conventional DC (cDC) cDC1 and cDC2 types which derive from a bone marrow common DC precursor and are present both in lymphoid organs and as interstitial DC in the parenchyma of non-lymphoid tissues such as skin, lung, gut, and liver (14). Comparative transcriptomic analyses pioneered by us and used by other groups, as well as functional studies, have demonstrated the existence of similar mononuclear phagocytes and DC subsets between human and mice (15–20). DC subset candidates have also been described in other mammals such as in ruminants and pigs. However, no systematic study has demonstrated the existence of a framework of homologous DC subsets throughout distant species [for review see Ref. (21)]. Overall, it remains unknown whether a similar diversity in mononuclear phagocyte subsets exists across distant mammals and vertebrates, and when during evolution this complex organization of the mononuclear phagocyte system arose.

The combination of phenotypic, functional, and ontogenic studies used in the mouse model cannot be used to define cell subsets in most other species of interest due to technical, financial, or ethical limitations. As the ontogeny and functions of cell types are instructed by specific gene-expression modules, cell type identity can be defined by its molecular fingerprinting (22). We thus reasoned that mononuclear phagocyte subset identity could be defined by gene-expression profiling, whatever the species. In addition, cell types that are homologous between species must exhibit closer molecular fingerprints and gene-expression programs than non-homologous cell types, based on the definition of homologous cell types as "those cells that evolved from the same precursor cell type in the last common ancestor" (23).

In this paper, we developed a streamlined approach (see Figure S1 in Supplementary Material) to identify homologous mononuclear phagocyte subsets in distant species with reference to the mouse, consisting in (i) designing antibody panels for sorting candidate cell subsets to high marker-based purity, (ii) generating genome expression profiling of the sorted cell subsets, and (iii) performing computational transcriptomic analyses to establish gene signatures and compare them to the transcriptomic fingerprints of the well-characterized immune cell types of the mouse referent species. Our analysis was extended to chicken cell subsets, showing that it is amenable to establish mononuclear phagocyte subset homology throughout vertebrates. We also derived gene-expression signatures and gene interaction networks that are selectively expressed in mononuclear phagocyte subsets in a conserved manner throughout distant mammals and that can be used to identify homologous subsets throughout species. The conserved gene-expression signatures and networks not only encompassed genes with known functions in mononuclear phagocyte subsets but also pointed out novel candidate genes likely involved in the ontogeny or functional specialization of these cell types. Finally, we conducted a phylogenetic analysis to examine the presence in bony fishes and in Lamprey of orthologs of genes from the transcriptomic signatures identified in mammals.

Materials and Methods

Pigs and Sheep for Blood Collection

All animal experiments were carried out under licenses issued by the Direction of the Veterinary Services of Versailles (accreditation numbers B78-93) and under approval of the Committee on the Ethics of Animal Experiments of AgroParisTech and INRA-Jouy-en-Josas (COMETHEA, authorization number 00604.01). The eight pigs (blood) used in this study (four males, four females) were around 2 years old and weighted between 60 and 85 kg. Down-sized pigs were kept at the Centre d'Imagerie Interventionnelle (Jouy-en-Josas). «Prealpe» female sheep (total 37, 50–80 kg), originate from and were raised in the «Unité Commune d'Expérimentation Animale» in Jouy-en-Josas, France. Blood (<400 ml/animal) was collected by venous puncture on sodium citrate.

Isolation of DC Subset Candidates, B Lymphocytes, and Mo from Pig Blood

PBMC were obtained from pig peripheral blood buffy coat samples by 1.076 g/ml density Percoll (GE Healthcare) gradient centrifugation (24). For B cell sorting, PBMC were surface-labeled with 2 μg/ml primary monoclonal antibody (mAb) against IgL (K139 3E1, IgG2a) followed by Alexa647-conjugated goat anti-mouse

IgG2a antibodies (Invitrogen). For pDC sorting, PBMC were surface-labeled with 2 μg/ml primary mAb anti-pig CD4 (PT90A, IgG2a), CD3 (8E6, IgG1), CD14 (CAM36, IgG1), and CD172A (74-22-15, IgG2b) followed by Alexa488, phycoerythrin (PE), or Alexa647-conjugated goat anti-mouse isotype-specific antibodies (Invitrogen). Blood pDC candidates were sorted as $CD3^-$ $CD14^-$ $CD4^+$ $CD172^{int}$ cells, based on previously published indicative data (25). For cDC candidates and Mo sorting, PBMC were surface-labeled with 2 μg/ml mAb anti-pig IgL (K139 3E1, IgG2a), anti-pig IgG (K138 4C2, IgM), anti-pig IgM (PG145A, IgM), anti-pig CD4 (PT90A, IgG2a), anti-human and pig cross-reacting CD14 (TUK4, IgG2a), anti-pig CD172A (74-22-15, IgG1), anti-artiodactyl MHC class II (Th21A, IgG2b), and chicken anti-human and artiodactyl cross-reacting CADM1 (3E1, IgY). The primary antibodies were revealed with Alexa488, PE, or Alexa647-conjugated goat anti-mouse isotype-specific antibodies and with donkey anti-chicken IgY Peridinin Chlorophyll Protein Complex (PerCP)-conjugated IgG. The cDC2 candidates were isolated as FSC^{hi} IgL^- IgG^- IgM^- $CD4^-$ $CD14^-$ MHC class II^+ $CADM1^-$ $CD172^{hi}$ or $CD172^{int}$ cells. The cDC1 candidates were isolated as FSC^{hi} IgL^- IgG^- IgM^- $CD4^-$ $CD14^-$ MHC class II^+ $CADM1^+$ $CD172^{lo}$ cells. Mo candidates were sorted as MHC class II^- $CD172^{hi}$ cells. Non-relevant antibodies (IgG1, IgG2a, IgG2b, and IgM) were systematically used as controls to measure the level of non-specific background signal caused by primary antibodies. The cell subsets were sorted by flow cytometry on the ImaGif Cytometry platform using the analyzer-sorter MoFlo XDP cytometer and the Summit 5.2 software from Beckman Coulter (cytometric assessment of post-sort purity >98%). The numbers of DCs that were collected per pig lay between 2 and 3×10^5 for pDC, 25 and 47×10^3 for cDC1, 20 and 40×10^5 for cDC2 candidates.

Isolation of DC Subset Candidates from Sheep Blood and B Lymphocytes and Macrophages from Sheep Spleen

Sheep PBMC were loaded on 1.065 density iodixanol gradient (Optiprep, Nycomed Pharma) to isolate low density cells from blood. Sheep pDC candidates were isolated by flow cytometry as previously described (26). For isolating sheep cDC candidates, the low density PBMC from several sheep were reacted with anti-CD11c mAb (2 μg/ml, OM1 clone, IgG1) followed by a saturating concentration of pacific blue-labeled anti-mouse IgG donkey Fab (50 μg/ml). After extensive wash, cells were further incubated anti-CD172A mAb (2 μg/ml, ILA24, IgG1) followed by a saturating concentration of Alexa488-labeled anti-mouse IgG donkey Fab (50 μg/ml). After extensive wash, cells were incubated with 2 μg/ml primary mAbs anti-ruminant B cells (DU-204, IgM), CD11b (ILA130, IgG2a), TCR1γ/δ receptor (CC15, IgG2a), CD45RB (CC76, IgG1), and chicken anti-human and artiodactyl cross-reacting CADM1 (3E1, IgY). The IgM and IgG2a primary antibodies were revealed with PE-conjugated goat anti-mouse isotype-specific antibodies, the IgG1 anti-CD45RB primary antibody was revealed with Alexa647-conjugated goat anti-mouse IgG1 antibody, and the anti-CADM1 with anti-IgY PerCP-conjugated IgG. The cDC2 candidates were isolated by flow cytometry as B^- $CD11b^-$ $TCR1^-$ $CD45RB^-$ $CD11c^+$ $CADM1^{lo}$ $CD172^{hi}$ FSC^{hi} cells. The cDC1 candidates were isolated by flow cytometry as B^- $CD11b^-$ $TCR1^-$ $CD45RB^-$ $CD11c^+$ $CADM1^{hi}$ $CD172^{lo}$ FSC^{hi} cells. The numbers of DCs that were collected per sheep lay between 1 and 2×10^5 for pDC, around 600 for cDC1, and around 4000 for cDC2. The far lower amounts of collected blood cDCs from sheep as compared to pig may probably originate from the multiple staining steps due to the necessity to separately identify several IgG1 as primary antibodies. B cells and MP were sorted by flow cytometry from isolated sheep spleen cells using the anti-ruminant B cell (DU-204, IgM) and anti-CD14 (CAM36, IgG1), respectively.

Production of Sheep MoDC

Three independent cultures of sheep MoDC were produced with GM-CSF as previously described (27).

RNA Extraction and Hybridization on Microarrays

Total RNA from subsets was extracted using the Arcturus PicoPure RNA Isolation Kit (Arcturus Life Technologies) and checked for quality with an Agilent 2100 Bioanalyzer using RNA 6000 Nano or Pico Kits (Agilent Technologies). All RNA samples had an RNA integrity number (RIN) above 8.5. When insufficient total RNA amounts for hydridization were obtained (<25 ng for sheep DNA chips, <50 ng for pig DNA chips), the RNAs from the sorted subsets of distinct animal were mixed. RNA amplification and labeling was performed using the one-color Low Input Quick Amp Labeling kit (Agilent Technologies) following the manufacturer's recommendations. Each RNA sample (25 ng for sheep and 50 ng for pig) was amplified and cyanin 3 (Cy3) labeled, and subsequently the complementary RNA (cRNA) was checked for quality on a Nanodrop and on an Agilent 2100 Bioanalyzer. The cRNAs (600 ng) were fragmented and used for hybridization on custom-designed Agilent ovine and porcine arrays. Our arrays for sheep and pig were custom-designed based on the commercial ovine Agilent arrays for these two species, as previously described (28, 29). In brief, the commercial probes with poor Sigreannot scores (30) were replaced with new probes designed using the e-array software from Agilent Technologies and including ovine or porcine orthologs of genes known to be selectively expressed in human and mouse DC subsets (15). After hybridization of the cRNAs on the custom-designed ovine array, the chips were washed according to the manufacturer's protocol and scanned using a G2565CA scanner (Agilent Technologies) at the resolution of 3 μm. The resulting .tiff images were extracted using the Feature Extraction software v10.7.3.1 (Agilent Technologies), using the GE1_107_Sep09 protocol. All the protocols used can be obtained by contacting the CRB GADIE facility[1]. The transcriptomic data from the chicken immune subsets were obtained from a previous study (31). All microarray data have been deposited in the Gene Expression Omnibus (GEO) database under reference numbers GSE9810, GSE53500, GSE55642 which have already been released and GSE66311 which is under embargo until publication of the present study.

[1] http://crb-gadie.inra.fr/

Computational Pipeline to Assess Cell Subset Homology Across Species

We have designed a computational pipeline in order to define cell subset homology across species, based on the analysis of gene-expression microarray data. In the current study, it has been applied to identify homology relationships between mononuclear phagocyte cells in mammalian species and then extended to the comparison with a more distant species (chicken). However, it can be applied to any cell type and to any species, provided that the annotations of the genes for each species are sufficiently well documented to allow the retrieval of the orthologous genes. In order to perform the comparison of expression profiles of cells coming from different species, thus from different platforms, we have designed two independent procedures. The first procedure (Figure S2A in Supplementary Material) is based on the assessment of the conservation of cell-specific fingerprints/signatures, as assessed by performing gene set enrichment analyses (GSEA, see below) between pairs of cell types. The second procedure (Figure S2B in Supplementary Material, see below) consists in cross-normalizing the expression datasets coming from the different species, in order to simultaneously examine the relationships between all cell types together.

Cross-Species Transcriptome Comparison by Pairwise Gene Set Enrichment Analyses

The methodological pipeline is depicted in Figure S2A in Supplementary Material, based on an example with comparison of three different species (A, B, and C). Species A is the reference species, i.e., the species for which the cell types are the most accurately described and generally also for which gene orthologous relationships can be retrieved from (mouse or human here). Species B and C are the test species, i.e., the species for which the identity of the cell types has to be established. Coming from three different platforms, the expression datasets have different numbers of probes, illustrated by boxes of different size. In brief, the strategy is to examine by GSEA whether the transcriptomic fingerprint of a given cell type (X) from the referent species A is enriched in one cell type (Y) of a test species (B for example) as compared to all other cell types of the same species. If this is the case, this would support the hypothesis that the cell type Y from the test species B is homologous to the cell type X of the referent species A. To perform these high-throughput GSEA in a processive way that could be easily reproduced and interpreted by other researchers devoid of bio-informatics expertise, we designed and implemented a dedicated software, called Bubble GUM (manuscript in preparation in which an extensive description of the software will be provided)[2]. Bubble GUM encompasses two main modules, GeneSign and BubbleMap, respectively, dedicated to the generation of gene sets and to their use for GSEA applied to multiple pairwise comparisons of samples integrated together into a simple graphical output that helps in the interpretation of the results. The first step consists in extracting from the reference species the transcriptomic fingerprints of each cell type. A cell-specific transcriptomic fingerprint can be defined as the list of genes that are more highly expressed in the cell type of interest than in all other cell types. These fingerprints were extracted using the "Min (test) vs. Max (ref)" method [(minimum expression among all replicates for all samples for which the transcriptomic fingerprint is defined/maximum expression among all replicates used as reference) ≥1.5-fold] (15, 32), using the GeneSign module of Bubble GUM. These transcriptomic fingerprints, in gene symbol format, will be assessed for enrichment on the expression datasets of species B and C. Thus, it is necessary to convert the probe annotations from the arrays of species B and C into the gene symbol of their orthologous counterparts in species A. For this purpose, we used the orthology relationships defined by the Sigenae pipeline which annotated the pig and sheep genes with their human and mouse orthologous gene symbols (30). The genes present on the gene chips of species B and C that were not associated to an orthologous counterpart in species A remained annotated with the gene symbol corresponding to their species of origin. The statistical enrichment of the cell-specific transcriptomic fingerprints extracted from the reference species A were then calculated between pairwise comparisons of cell types from species B or C with the GSEA methodology, using gene set permutations for computing the *p*-values and false-discovery rates (FDR) (33). This was achieved, and the results graphically represented, by using the BubbleMap module of Bubble GUM.

Cross-Normalization of the Species-Specific Expression Datasets

Using the same starting expression datasets, this is an alternative strategy which is complementary to the pairwise GSEA of the species-specific expression datasets, since it allows clustering all cell types together based on the overall evaluation of the proximity of their expression patterns of hundreds to thousands of orthologous genes. The first step (Orthology Filter) consists in aligning the genes across the species (A, B, and C). It requires retaining only one representative probe per gene for each species/platform. This is needed since, in microarray designs, many genes are often each represented several times by a number of individual probes having each a different signal-to-noise ratio. However, probes have no equivalence across species, whereas genes do. In our experience, the signal-to-noise ratio is generally better for probes that have the strongest signal in positive control samples, while certain probes that have a low signal-to-noise ratio can give misleading high fold changes across conditions when using a limited number of replicates. Hence, we computed for each probe in each platform the sum of normalized expression values across all samples and kept for each gene the probe that had the highest computed value. Then, for the genes of species B and C, we retrieved the gene symbol of their orthologous counterparts in species A (reference species). In the example illustrated in Figure S2B in Supplementary Material, species A is the reference: the genes of species B and C are thus annotated using the gene symbol of the orthologous genes in the species A. The genes not represented in each of the gene chip platform used were removed from the analysis. This Orthology Filter yielded a filtered expression dataset for each species, where the number of genes and their associated symbols were similar between all species, as illustrated by boxes of the same size (Figure S2B in Supplementary Material). In order to be able

[2]http://www.ciml.univ-mrs.fr/applications/BubbleGUM/index.html

to rigorously merge the different datasets together, the dynamic ranges of expression values for each gene across all species must be homogenized by setting for each dataset and for each gene across all samples the mean expression to 0 and the variance to 1, a process called data centering and reduction. To prevent this mathematical transformation of the expression data to introduce noise by forcing artifactual expression changes for genes that were unregulated in the initial datasets, it is mandatory to remove all the genes that are not regulated in at least one of the datasets. This thus requires keeping only the genes that are differentially expressed between at least two cell types for each of the species studied. This was achieved in the second and third steps of the data processing. The second step (Differentially Expressed Gene, DEG, Filter) consisted in identifying independently in each dataset the genes that are differentially expressed between at least two cell types. The identification of DEG was performed by calculating the minimal ratio between each pairwise comparison of cell types and by selecting only the genes for which this minimal ratio was higher than twofold. The third step (DEG intersection) consisted in keeping only the genes that were common to all filtered DEG lists, i.e., the orthologous genes which expression was modulated across samples in each of the species studied. The fourth step consisted in data centering and reduction for each dataset, which was performed using the R statistical environment. This step consists in setting, for each dataset, the mean to 0 and the variance to 1, so that all datasets are comparable. In the fifth and final step, the different datasets were merged together simply by aligning their rows based on the common gene symbol extracted from species A. The final cross-normalized expression dataset including the data for all species was then used to perform canonical analyses for classification of samples, namely here hierarchical clustering.

Generation of Conserved Cross-Species Cell Type-Specific Signatures

For each species (human, mouse, sheep, and pig), the transcriptomic fingerprint of each cell type was generated by selecting the genes more highly expressed in the cell type of interest, as compared to all other studied cell types of the same species in the case of "absolute" transcriptomic signatures, or as compared to selected cell populations of the same species in the case of "relative" transcriptomic signatures, using the "Min (test) vs. Max (ref) ≥1-fold" method. Once the fingerprints had been obtained for each species for a given cell type, the gene identifiers were all converted into their corresponding official human gene symbol using BioMart and we selected the intersection of these four lists as the final conserved cross-species transcriptomic signature specific of that cell type, with the following exceptions. First, for certain cell types such as MoDC, data were available from only three, and not four, species. Second, in order to avoid removing putatively relevant signature genes, we kept in these signatures the genes found in all species but one, when their absence in the signature of that given species was due to absence or non-functionality of corresponding ProbeSets on the array of that species.

Real-Time PCR

For relative quantitation of gene expression in subsets, RNA was reverse transcribed using random primers and the Multiscribe reverse transcriptase (Applied Biosystems). Real-time PCR (qPCR) was carried out with 300 nM primers in a final reaction volume of 25 µl of 1 X SYBR Green PCR Master Mix (Applied Biosystems). The primers used to amplify ovine and porcine cDNA were designed with the Primer Express software (v2.0) using publically available GenBank sequences (Table S1 in Supplementary Material). PCR cycling conditions were 95°C for 10 min, linked to 40 cycles of 95°C for 15 s and 60°C for 1 min. Real-time qPCR data were collected by the Mastercycler® e0p realplex-Eppendorf system and $2^{-\Delta Ct}$ calculations for the relative expression of the different genes (arbitrary units) were performed with the Realplex software using GAPDH for normalization. All qPCR reactions showed >95% efficacy.

Results

Isolation of Mononuclear Phagocyte Subset Candidates from Artiodactyl Blood or Spleen Using a Set of Surface Markers

In order to establish a framework of homologous mononuclear phagocyte subsets across different species, we selected two mammalian artiodactyl species, sheep and pig, belonging to the Laurasatherians, a phylogenetically distant order from the Euarchontoglires that include the human and mouse species (**Figure 1**). A set of available antibodies exist to isolate cell subsets in these species of interest as food animals, hosts of zoonotic diseases, and biomedical models. We focused on blood or spleen immune subsets, because (i) a large source of transcriptomic data is available from this compartment in the human and mouse reference species, (ii) they are readily accessible with a minimum of technical biases in all species, and (iii) their gene-expression profiles are not expected to be influenced by peripheral tissue imprinting. We designed antibody panels to sort the subsets. In human and mice, cDC lack expression of T and B lymphocyte and Mo/MP markers and they abundantly express CD11c and MHC class II. Independent groups identified SIRPα as a conserved marker suitable to distinguish cDC2 from cDC1 across species (17, 34). Whereas XCR1 stands as the best marker for identifying cDC1 (34–41), appropriate reagents are not yet available in species outside human and mouse, and CADM1, whose sequence is highly conserved in evolution (42), can be used as an alternative (43, 44). cDC1 and cDC2 candidates were isolated from sheep and pig low density blood cells after exclusion of irrelevant cells (**Figure 2A** for sheep and **Figure 2B** for pigs, see Material and Methods section). The «candidate» nature of a sorted cell subset is marked by a star before the considered subset name in this paper. Due to restricted reagent availability, CD11c and MHC2 class II markers were used to isolate sheep and pig cDC, respectively.

In the case of pig, two populations being $CADM1^{-}$ $CD172^{+}$ or $CD172^{int}$ were identified and selected as potential candidates and designated as *cDC2 and **cDC2, respectively (**Figure 2B**). We previously published the marker phenotype, morphology, and type I IFN production properties of sheep lymph and blood *pDC as $CD45RB^{+}$ FSC^{high} $TCR\gamma/\delta^{-}$ B^{-} $CD11b^{-}$ cells (26, 48). The sorted cells were very potent at type I IFN production upon viral-type stimulation, demonstrating at the functional level that they were highly enriched in pDC. Moreover, the sorted cells

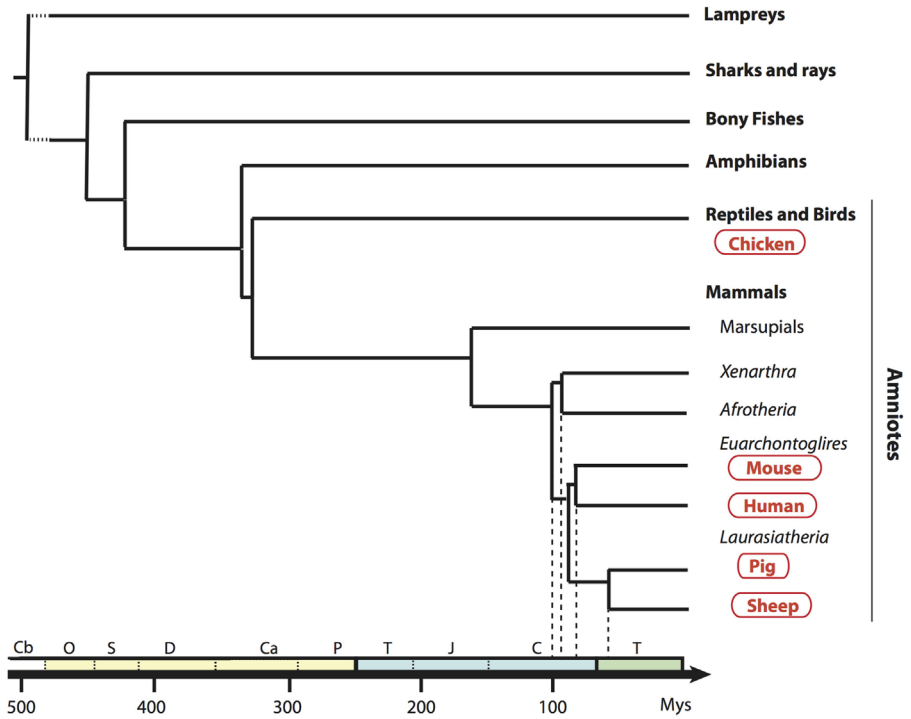

FIGURE 1 | Phylogenetic tree of a broad selection of vertebrates. Phylogenic relationships and divergence time of clades are drawn according to Hajjoubi et al. (45), de Jong et al. (46), and Douady et al. (47). Geological periods are indicated at the bottom in the following order: primary era in yellow with Cambrian (Cb), Ordovician (O), Silurian (S), Devonian (D), Carboniferous (Ca), Permian (P); secondary era in blue with Trias (T), Jurassic (J), Cretacean (C), and the tertiary era (T) in green.

had the expected size and plasmacytoid morphology, indicating that they were not contaminated by other types of myeloid cells (48). Blood *pDC from pigs were sorted as CD3$^-$ CD4$^+$ CD172^{dim} cells based on marker phenotype, morphology, and type I IFN production properties established by others (25, 49). Pig *Mo were sorted as CD172^{high} MHC class II$^-$ cells and sheep splenic *MP as CD14$^+$ cells.

To decrease the risk of improper identification of sorted cell subsets, we performed a quality control consisting in examining the expression of a few control genes by qRT-PCR (**Figure 2C**) prior to performing genome-wide transcriptomic analyses. Control genes were chosen based on their high selective expression in a given subset of mononuclear phagocytes in a conserved manner between mouse and human (15, 36) and encompassed *TCF4* for pDC, *CD14* for Mo/MP, *FLT3* for cDC and pDC, *ZBTB46* for cDC, *BATF3* and *XCR1* for cDC1. As expected, *TCF4* was expressed to much higher levels in sheep (26) and pig *pDC as compared to all other cell types examined except for pig **cDC2. *CD14* was expressed at much higher levels in sheep and pig *mono/MP as compared to all other cell types examined except one of the two replicates of pig *cDC2. *FLT3* was expressed at much higher levels in sheep and pig *cDC1 and in sheep *cDC2 as compared to all other cell types examined. *BATF3* and *XCR1* were expressed at higher levels in sheep and pig *cDC1 as compared to all other cell types examined. Importantly, these control analyses have allowed us to improve our initial strategy for sheep *cDC1 and *cDC2 sorting. In fact, in our initial sorting (Figure S3 in Supplementary Material), the CD45RB$^+$ cells were not excluded to sort cDC candidates, and the *cDC1 were found to express high levels of *TCF4* mRNA, leading us to refine the sheep cDC sorting as presented in **Figure 2**. Thus, overall these control analyses validated our strategy for phenotypic identification and flow cytometry purification of sheep and pig *pDC, *Mo/MP and *cDC1 DC, and of sheep *cDC2. In the case of pig cell subsets, the nature of **cDC2 and *cDC2 was not clear since the former expressed high levels of *TCF4* and *XCR1*, and the latter expressed relatively high levels of *CD14* in one out of two replicates. Because pig **cDC2 presented a relatively high expression level of both *TCF4* and *XCR1*, we concluded that they were significantly contaminated by pDC and cDC1. Therefore, we excluded these cells from further analyses and assumed that pig *cDC2 cells were the proper candidate.

Use of Pairwise Gene Set Enrichment Analyses for Assessment of the Similarity Between Mononuclear Phagocyte Subsets Across Distant Mammal Species

As a first approach to establish mononuclear phagocyte subset homology across species, we determined the level of similarity between artiodactyl, mouse, and human mononuclear phagocytes using pairwise GSEA, as previously performed to characterize

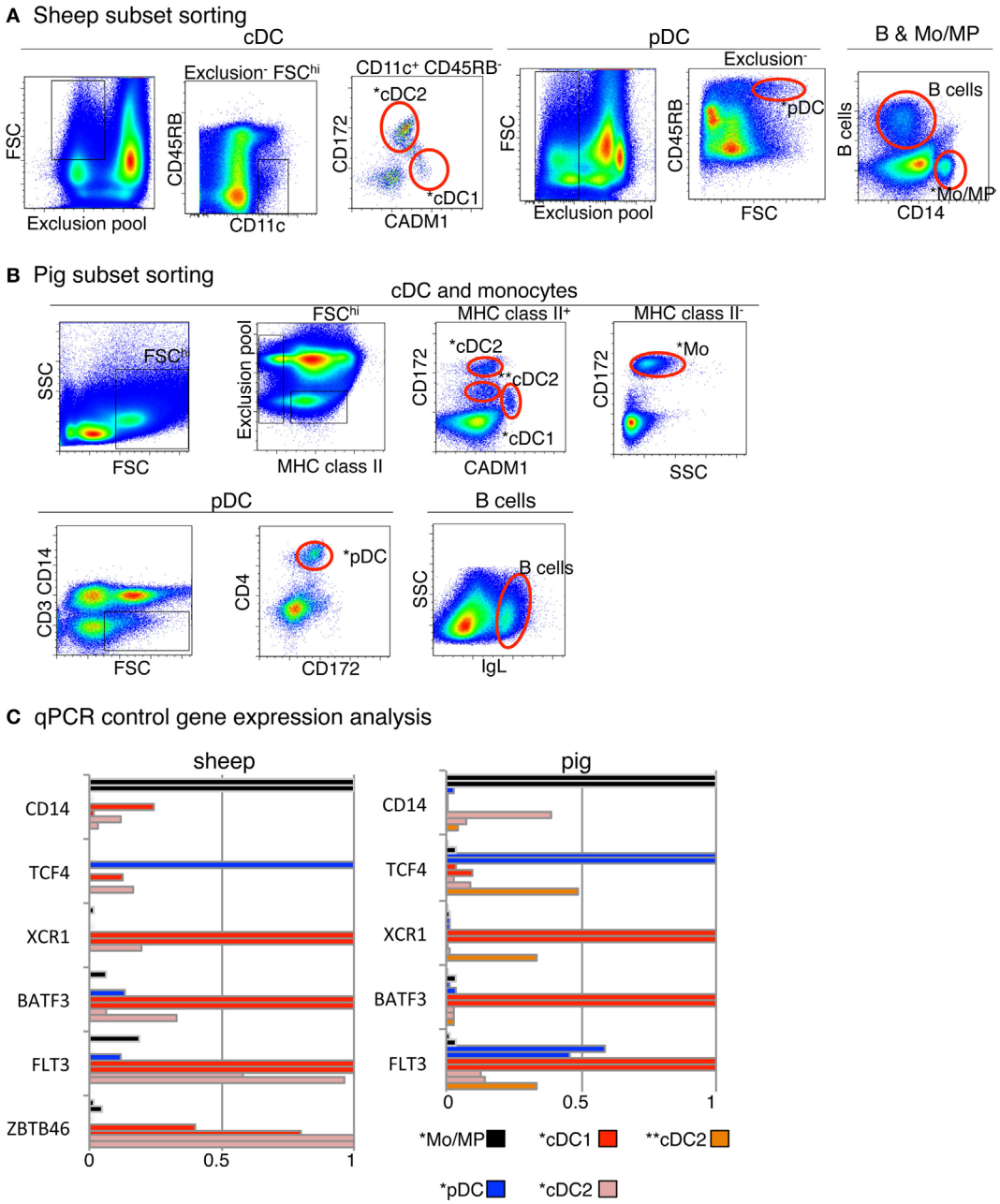

FIGURE 2 | Sorting of B cells, DC subset candidates, and Mo/MP candidates from pig and sheep blood or spleen and analysis of their expression of control genes. **(A)** Sheep cell subset sorting from blood and spleen. For sorting of blood cDC subset candidates, low density blood cells were gated on FSC^{hi} CD11c^{+} B^{-} CD11b^{-} TCR1^{-} CD45RB^{-} cells and analyzed for CADM1 and CD172 expression, based on isotype control references for each staining. The CADM1^{hi} CD172^{lo} (*cDC1) and CADM1^{lo} CD172^{+} (*cDC2) cells were sorted. Blood pDC candidates (*pDC) were sorted as low density FSC^{hi} B^{-} CD11b^{-} TCR1^{-} CD8^{-} CD11c^{-} CD45RB^{+} cells. Splenic candidate *Mo/MP were sorted as CD14^{+} cells. Splenic B cells were identified as DU-2-104^{+} cells. **(B)** Pig cell subset sorting from blood. For cDC candidate sorting, low density PBMC were gated on FSC^{hi} MHC class II^{+} B^{-} CD14^{-} CD4^{-} cells and analyzed for CADM1 and CD172 expression. One cDC1 candidate population was identified and sorted, as CADM1^{+} CD172^{lo} (*cDC1). Two cDC2 candidate populations were identified and sorted, as CADM1^{-} CD172^{hi} (*cDC2) and CADM1^{-} CD172^{int} (**cDC2). Candidate Mo were sorted as CD172^{+} MHC2^{-} cells (*Mo). Candidate pDC were sorted as CD3^{-} CD14^{-} CD4^{+} CD172^{int} cells (*pDC). B cells were identified and sorted as IgL^{+} cells. **(C)** qPCR analysis of the expression of control genes in sorted candidates from one or two animals. RNA from candidate cell subsets (left, sheep; right, pig) were subjected to detection of control transcripts by qPCR. Control transcripts were chosen based on their high selective expression in specific subsets of mononuclear phagocytes in a conserved manner between mouse and man, i.e., *TCF4* for pDC, *FLT3*, *BATF3* and *ZBTB46* for cDC, *XCR1* for cDC1, and CD14 for Mo/MP. Data are represented as relative expression levels normalized to maximal expression across cell types, each bar corresponding to a distinct animal.

human immune cell subsets (19) and chicken cDC (31). To that aim, we used publicly available transcriptomic data from a selection of human and mouse immune cell types (Data Sheet S1 in Supplementary Material). We established human and mouse transcriptomic fingerprints for B cells, pDC, cDC1, Mo/MP, MoDC, cMo, and ncMo as the list of genes that are expressed at least 1.5-fold higher in the index cell population than in a large number of other immune cell types (Data Sheet S2 in Supplementary Material). B lymphocytes were chosen in all species as a reference cell subset, because their phenotypic identification in each species and their homology across species are already well established, and because they are expected to share with mononuclear phagocytes a genetic program underlying their common function of antigen-presenting cells. We generated a common fingerprint for Mo and tissue MP because their gene program is very close in the mouse (9), even though tissue MP generally derive from embryonic precursors rather than from circulating blood Mo. We could not establish a human or mouse cDC2 transcriptomic fingerprint with a sufficiently large number of genes for subsequent reliable statistical analysis. We also defined relative transcriptomic signatures for cDC vs. Mo/MP as the list of genes that are 1.5-fold higher in all cDC relatively to Mo and MP from different tissues, and reciprocally (Data Sheet S2 in Supplementary Material). Finally, we identified transcriptomic fingerprints from human and mouse MoDC (12). We then tested whether the transcriptomic signatures of mouse and human immune cell types were enriched between sheep or pig candidate cell subsets using GSEA (33) (**Figures 3** and **4**). As control, since homologies between mouse and human cell subsets have been previously demonstrated by other methods of transcriptional analyses (15, 16, 18), mouse fingerprints were also used for GSEA analysis on human cells (Figure S4 in Supplementary Material) and reciprocally (Figure S5 in Supplementary Material).

As expected, sheep B cells were significantly enriched for the expression of both human and mouse B cell transcriptomic fingerprints as compared to all other sheep cell subsets examined (**Figure 3**, ❶). The sheep *pDC were enriched for the human and mouse pDC fingerprints in most comparisons (**Figure 3**, ❷), suggesting that sheep *pDC correspond to homologs of human and mouse pDC. However, both mouse and human pDC fingerprints were not significantly enriched in the comparison of sheep *pDC with *cDC2 (NES = 1.29 and 1.24, and FDR = 1.0 and 1.0, respectively), indicating that sheep pDC probably contaminate sheep *cDC2 despite exclusion of $CD45RB^+$ cells for their purification. The sheep *pDC were also enriched for the human B cell fingerprint in most comparisons (except with sheep B cells), what can be partly explained by the known overlap between the gene-expression program of pDC and B (15, 50–52); however, the human pDC fingerprint is not enriched in the sheep *pDC comparison with B cells and the extent of the human B cell fingerprint enrichment in sheep *pDC is above the expectations provided by similar analyses in the human and mouse reference species (Figures S4 and S5 in Supplementary Material), all of this indicating that B cells are likely to contaminate sheep *pDC despite exclusion with a pan-B cell marker for *pDC selection. Finally, *cDC2 did not show a clear enrichment for any human and mouse signatures (**Figure 3**, ❸). However, it is also the case when examining enrichment of mouse cell subset fingerprints in human cDC2 (Figure S4 in Supplementary Material, ❸) and reciprocally (Figure S5 in Supplementary Material, ❸). Hence, this GSEA approach is not very informative for identification of cDC2, due to the lack of robust human or mouse fingerprints that are specific of this cell type as mentioned earlier. Sheep *cDC1 were significantly enriched in the human and mouse cDC1 fingerprints in all comparisons (**Figure 3**, ❹). They were also enriched systematically in the mouse cDC vs. Mo/MP fingerprints. This suggested that sheep *cDC1 correspond to true homologs to human and mouse cDC1. Sheep splenic *Mo/MP were strongly enriched for the human and mouse Mo/MP vs. cDC fingerprints except when compared to MoDC, and not for the human and mouse fingerprints of B lymphocytes, pDC, or cDC (**Figure 3**, ❺). This confirmed that sheep splenic *Mo/MP belong to the monocytic lineage and not to the B nor DC lineages. However, their precise identity remained unclear as they were enriched for the mouse cMo fingerprint but not for the human cMo or ncMo fingerprints. When mouse fingerprints were applied on human immune cell subsets comparisons and vice versa, there was also no consistent alignment of ncMo between the two species (Figures S4 and S5 in Supplementary Material, highlights ❺ and ❻). Finally, sheep *MoDC that were derived from bone marrow cells in GM-CSF (27), were systematically and strongly enriched in the human and mouse MoDC signatures (**Figure 3**, ❻), confirming the homology between these three populations.

A similar analysis for pig candidate cell subsets also clearly established similarities with their putative human and mouse equivalents for B cells (**Figure 4**, ❶), pDC (**Figure 4**, ❷), and cDC1 (**Figure 4**, ❹) but not for cDC2 (**Figure 4**, ❸). Pig *Mo were clearly enriched for human and mouse fingerprints of cells of the monocytic lineage, and not for human and mouse signatures of B lymphocytes, pDC, or cDC (**Figure 4**, ❺).

Thus, altogether, GSEA analysis of the sheep and pig data for the fingerprints of human and mouse immune cell subsets gave results as informative as those obtained when comparing together human and mouse cell types, and clearly established similarities between sheep and pig cell subset candidates and their putative human and mouse equivalents for B cells, pDC, cDC1, and MoDC. Further analyses are necessary to precisely identify the nature of sheep and pig *cDC2 and *Mo/MP subsets.

Confirmation and Extension of the Conclusions on the Similarity Between Mononuclear Phagocyte Subsets Through Global and Simultaneous Analysis of the Gene-Expression Profiling of All Cell Types from Mammalian Species Using Hierarchical Clustering

In order to confirm the identification of homologous mononuclear phagocytes across species as deduced from GSEA analyses, and to potentially gain more insights into the exact nature of pig and sheep *cDC2 and *Mo/MP, we next processed all the data together for global analysis by hierarchical clustering (**Figure 5**). Only the genes that showed significant variation in their expression across subsets in each species were selected

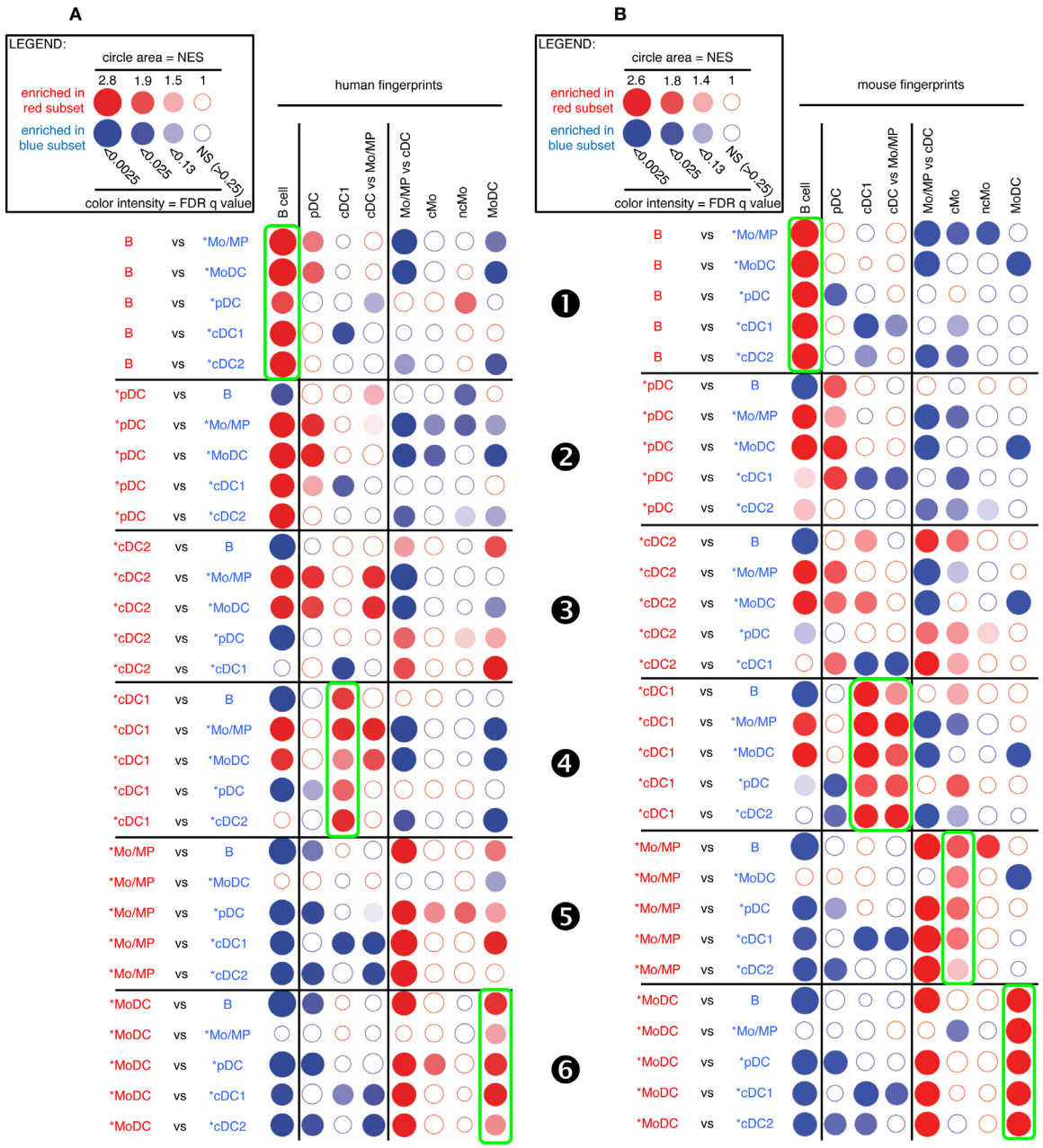

FIGURE 3 | GSEA-based assessment of the identity of sheep cell subset candidates by comparison with well-defined human and mouse mononuclear phagocytes. Candidate sheep cell subsets were compared to one another for their relative enrichment in transcriptomic fingerprints (GeneSets) specific of human **(A)** or mouse **(B)** mononuclear phagocyte subsets, using GSEA through the Bubble GUM software. The human and mouse GeneSets were defined through the same approach based on pre-existing knowledge of equivalency between human and mouse mononuclear phagocytes. A GeneSet specific for B cells was included as a control for the methodology, since the identity of this cell type is clearly established in all species and its homology across species is undisputed. The GeneSets used were named and defined as follows. The transcriptomic fingerprints "B cell," "pDC," "cDC1," "cMo," "ncMo," and "MoDC" consisted in the lists of human/mouse genes showing a high selective expression in the eponym human/mouse cell subset as compared to many other leukocytes (see Materials and Methods for further details, Data Sheet S2 in Supplementary Material). The transcriptomic fingerprints "cDC vs. Mo/MP" and "Mo/MP vs. cDC" consisted in the lists of human/mouse genes expressed in cDC to higher levels than in Mo/MP, and reciprocally (Data Sheet S2 in Supplementary Material). All possible pairwise comparisons between sheep cell subsets were performed to assess their respective expression of the transcriptomic fingerprints of human and mouse mononuclear phagocyte subsets, using the Bubble GUM software for calculations and graphical output. Results are represented as bubbles, in a color matching that of the cell subset in which the GeneSet was enriched. Stronger and more significant enrichments are represented by bigger and darker bubbles, as illustrated in the legend box of the figure. Specifically, the surface area of bubbles is proportional to the absolute value of the normalized enrichment score (NES). The color intensity of dots is indicative of the false-discovery rate (FDR) statistical value.

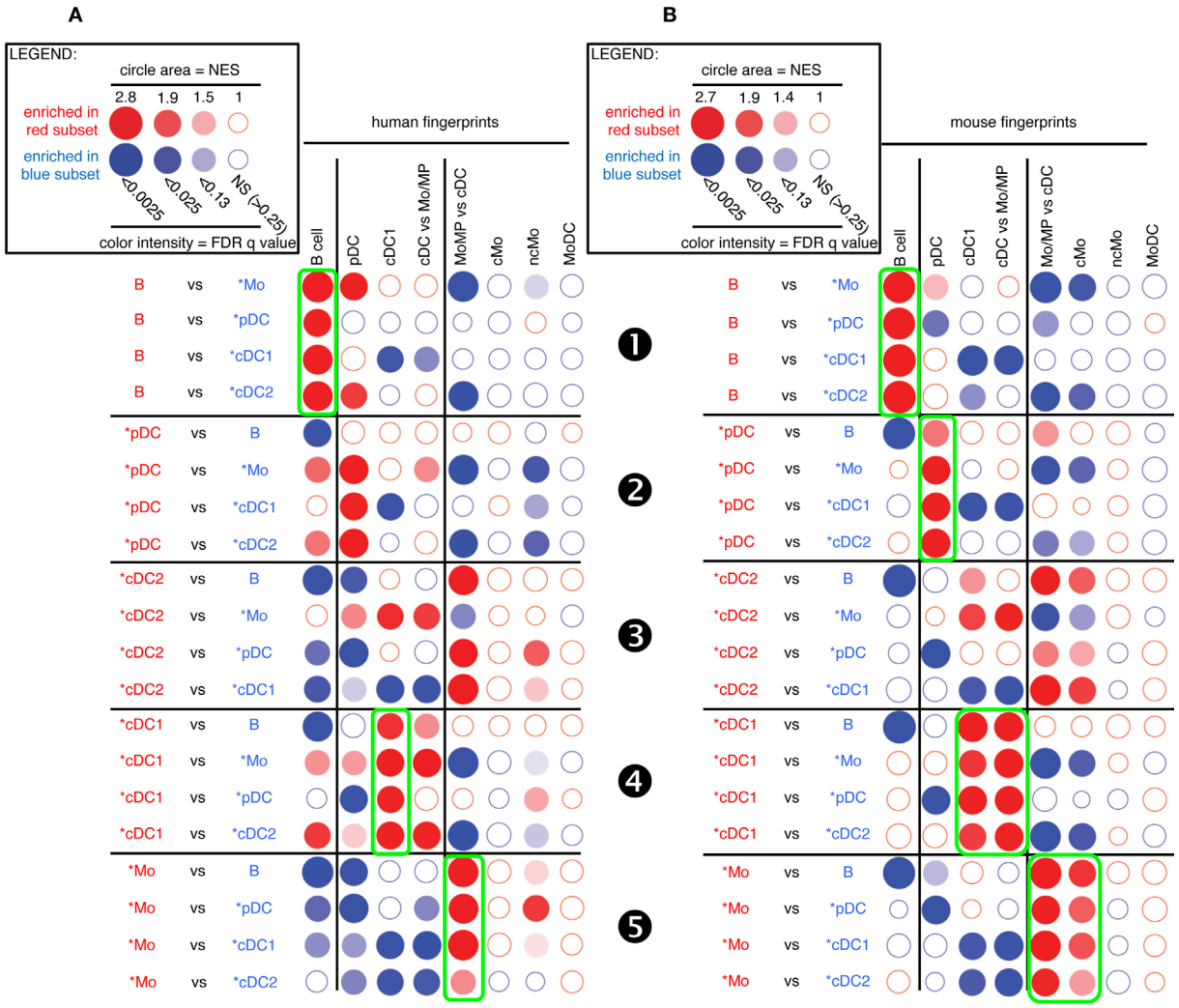

FIGURE 4 | GSEA-based assessment of the identity of pig cell subset candidates by comparison with well-defined human and mouse mononuclear phagocytes. The gene-expression data for pig cell subset candidates were analyzed exactly as described in **Figure 3** for sheep cell subset candidates. Candidate swine cell subsets were compared to one another for their relative enrichment in transcriptomic fingerprints specific of human **(A)** or mouse **(B)** mononuclear phagocyte subsets, using GSEA through the Bubble GUM software.

and the resulting datasets were normalized across species. All B cells from the four mammalian species grouped together in a specific branch of the tree, rather than each with other immune cells of the same species. This finding validates hierarchical clustering as an alternative method for identifying homologous mononuclear phagocytes across species. A closer examination of the dendrogram shows that the different cell types grouped in two major branches. The first one encompassed all the known and candidate cells of the monocytic lineages and pig *cDC2, and split further into two subgroups, one including all the identified or candidate MoDC, and the other one including all the identified or candidate Mo/MP and pig *cDC2. The second branch encompassed all the other cell types known or hypothesized not to belong to the monocytic lineage. This branch further split into two sub-branches, one constituted of the group of B cells and of the group of identified or candidate pDC, and the other constituted of identified or candidate cDC subsets except pig *cDC2. The common clustering of B and pDC transcriptome can be explained by the shared gene-expression program between B and pDC as mentioned above. Hence, this analysis confirmed the conclusion already drawn from the GSEA analyses, namely the monocytic nature of sheep and pig *Mo/MP and *MoDC, as well as the homology between pig, sheep, mouse, and human *pDC/pDC. Moreover, the hierarchical clustering analysis allowed to better define the nature of sheep and pig *cDC2. Specifically, it confirmed the hypothesis that sheep *cDC2 belong to the cDC family, while, on the contrary to our *a priori* assignment, it shows that pig *cDC2 rather resemble Mo than cDC. However, within the branch of monocytic cells, this analysis grouped Mo/MP by species of origin rather than by cMo vs. ncMo subsets. Similarly, this analysis grouped cDC by species rather than by cDC1 vs. cDC2 subsets.

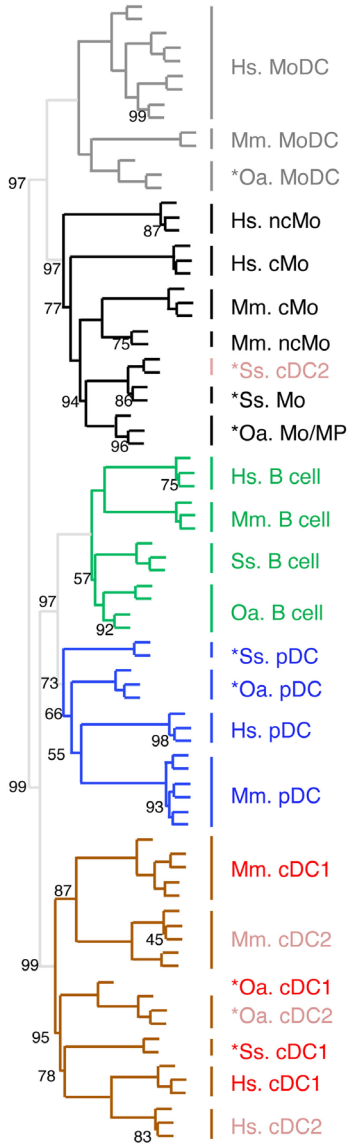

FIGURE 5 | Confirming and completing homology assignment of sheep and pig candidate Mo/MP, pDC, and cDC by unsupervised hierarchical clustering with human and mouse cell types. The datasets of each species were filtered and cross-normalized in order to allow mixing them all together for global analysis of the relationships between sheep, pig, mouse, and human mononuclear phagocyte subsets by using unsupervised hierarchical clustering. In brief, this analysis is focused on 1926 unique orthologous genes (i) for which a functional and specific ProbeSets was present on the microarrays for each species and (ii) which were found to be differentially expressed in each species between at least two subsets of mononuclear phagocytes. For each species and each of these 1926 genes, the expression data was then transformed to a mean = 0 and a variance = 1, in order to cross-normalize expression values to a similar dynamic range between the different datasets. For each cell type, the initials of the scientific name of the species of origin are indicated as a prefix: Hs, human; Mm, mouse; Ss, pig; and Oa, sheep. The robustness of the tree was tested by multiscale bootstrap resampling using Pearson's correlation as distance and average linkage as cluster method, with 1000 iterations at 10 different dataset sizes comprised between 50 and 140% of the complete dataset. An AU (approximately unbiased) *p*-value (percentage) was calculated and placed on the nodes of the cluster dendrogram. Missing percentages correspond to 100%.

Identification of Similarity Between Subsets of DC and of Mo Across Species Through Hierarchical Clustering Analyses Focused on These Cell Types

The expression patterns of genes outside of the cell types of interest may mask similarity between cDC or Mo subsets, as previously reported (15). Hence, we further evaluated the similarities between subsets of cDC on the one hand, and of Mo/MP on the other hand, by re-analyzing their gene-expression profiles focusing only on the genes that showed significant variation in their expression across DC subsets (**Figure 6**) or Mo/MP (**Figure 7**) in each species. Pig data were not used in the analysis focused on cDC, because, pig *cDC2 belonged to the monocytic branch and not to the DC branch of **Figure 5**. Sheep data were not used in the analysis focused on Mo/MP, because only one subset of sheep Mo/MP had been purified. Remarkably, these focused analyses grouped samples by cell types rather than by species. The cDC-focused hierarchical clustering confirmed the conclusion drawn from GSEA that sheep, mouse, and human cDC1/*cDC1 are homologs, and refined our understanding of the identity of sheep *cDC2 by showing their homology to mouse and human cDC2 (**Figure 6**). The Mo/MP-focused hierarchical clustering allowed to newly identify pig homologs to mouse and human cMo vs. ncMo (**Figure 7**). Pig *cDC2 correspond to ncMo and pig *Mo correspond to cMo. In a complementary phenotypic FACS analysis, we confirmed that likewise human ncMo as compared cMo, pig *cDC2 express higher membrane levels of CD16 and CD163 as compared to pig *Mo (Figure S6 in Supplementary Material).

Altogether, our comparative analyses of the gene-expression profiles of mononuclear phagocyte subsets across mammals

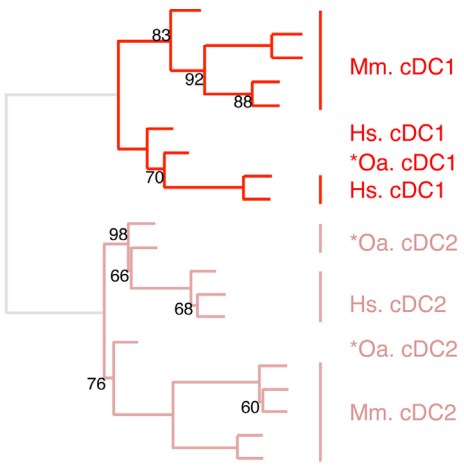

FIGURE 6 | Confirming homology assignment of sheep cDC1 and cDC2 candidates by unsupervised cross-species hierarchical clustering focused on cDC subsets. An unsupervised cross-species hierarchical clustering analysis was performed as described in **Figure 5**, but focused only on cDC subsets. The corresponding filtered dataset included 868 unique orthologous genes found regulated between cDC1 and cDC2 from human (Hs), mouse (Mm), and sheep (Oa). Pig cDC could not be included in this analysis due to the lack of data on proper pig cDC2.

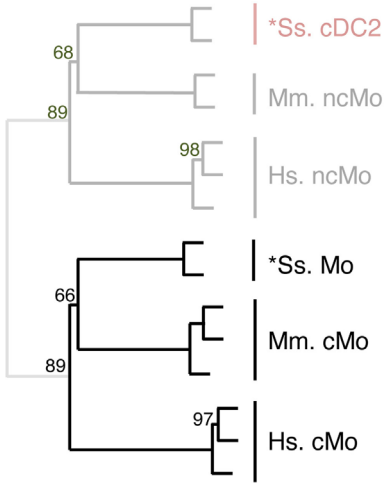

FIGURE 7 | Completing homology assignment of pig cDC2 DC candidate to non-classical Mo subset by unsupervised cross-species hierarchical clustering focused on Mo subsets. An unsupervised cross-species hierarchical clustering analysis was performed as described in **Figure 5**, but focused only on cells from the monocyte branch of the tree obtained in **Figure 5**. The corresponding filtered dataset included 191 unique orthologous genes found regulated between cMo and ncMo from human (Hs), mouse (Mm), and pig (Ss). Sheep data could not be included in this analysis due to lack of data on subsets of sheep monocytes.

indicated that the complex specialization of these cells into distinct subsets is conserved across mammals for both DC and Mo. Subset grouping did not indicate existence of a relationship between transcriptomic proximity of subsets and phylogenetic closeness of species. The conserved organization across distant mammals suggests that the mononuclear phagocyte complexity arose in a common mammalian ancestor and that the different subsets can be considered as homologous subsets across mammals.

Evidences for Homologous cDC and Mo/MP Lineages Across Warm-Blooded Vertebrates

We recently generated the transcriptomic profile of MP, total cDC, and B cells from chicken spleen and found similarities with human and mouse corresponding immune cell subsets by GSEA (31). In order to extend our subset homology analysis to non-mammalian vertebrates, we normalized and processed the transcriptomic data in a hierarchical clustering analysis as described above, using mammalian and chicken Mo/MP, B cells, and cDC subsets (**Figure 8**). There again, a tree consisting of two main branches was obtained, corresponding to a split between Mo/MP and B cells/DC. In the cDC branch, the cDC1 subset clustered together and included the chicken total cDC. The chicken MP grouped with the mammalian Mo/MP. Whereas this analysis is still partial due to limited knowledge and availability on marker sets for sorting immune cell subsets in chicken, it shows that our transcriptomic comparative approach can be used to define subset homology throughout vertebrates. It also further supports that separation of mononuclear phagocytes into Mo/MP and cDC occurred early during vertebrate evolution and must already have been in place in the common ancestor of reptiles (including birds) and mammals.

Identification of Mononuclear Phagocyte Gene-Expression Signatures Across Mammals

Taking advantage of our multi-species microarray data, we sought to identify core gene-expression signatures that should universally define at the molecular level each of the mononuclear phagocyte subset and that should hold biological relevance based on their selective and conserved expression in homologous subsets throughout mammalian evolution. Absolute signatures ["Min (test) vs. Max (ref)" method, see Materials and methods] encompassed all genes selectively expressed at higher levels in the cell subset of interest (index population) as compared to all the other cell subsets studied (comparator populations), in all species studied. An absolute signature was computed for B cells in order to validate the approach by comparison of the gene list obtained with the advanced knowledge available on the biology of this lymphocyte population. Absolute signatures were also found for pDC, cDC1, and MoDC. Relative signatures encompassed genes selectively expressed to higher levels in one or several cell subsets of interest (index population) as compared to a selection of other cell subsets (comparator populations). The choice of index and comparator populations was largely based on the branching of different cell subsets in hierarchical clustering (**Figure 5**), or on known sharing of specific functions between cell subsets in mouse or human. The conserved absolute and relative gene-expression signatures in mononuclear phagocyte subsets are listed in **Table 1** and Data Sheet S3 in Supplementary Material. In several instances, Ingenuity Pathway Analysis (IPA) mapped a high proportion of the genes to gene interaction networks (**Figure 9** for the DC lineage subsets, **Figure 10** for the monocytic lineage subsets and Figure S7 in Supplementary Material), and revealed predicted upstream regulators (**Figure 11A**) and canonical pathways and functions (**Figure 11B**) that are described thereafter for B cells, DC lineage subsets, and Mo/MP categories. Although certain functions or pathways were enriched in several gene signatures, the genes responsible for the enrichments differed (Data Sheet S4 in Supplementary Material) and pointed out to different, complementary contributions of the distinct cell types to the corresponding functions or pathways.

The conserved B cell signature that we use as our reference subset (**Table 1**) includes a regulatory gene network directed to immunoglobulin production (Figure S7 in Supplementary Material), with PAX5 as an upstream regulator ($p = 10^{-5.8}$) (**Figure 11A**). SOX11 ($p = 10^{-8}$) and FOXO1 ($p = 10^{-7}$) are predicted to be other upstream regulators in the conserved B signature (**Figure 11A**), in agreement with existing knowledge. As expected, this signature is associated to B lymphocyte ontogeny and functions [e.g., "development of B lymphocytes" $p = 10^{-10.4}$, "antibody response" ($p = 10^{-7.5}$), "proliferation of B lymphocytes" ($p = 10^{-10.5}$), and "morphology of B lymphocytes" ($p = 10^{-7.5}$) as well as to the "B cell receptor signaling" pathway ($p = 10^{-7.3}$)] (**Figure 11B**). The B cell signature also pinpoints to genes without any known function in B cells yet, such as the cell cycle gene

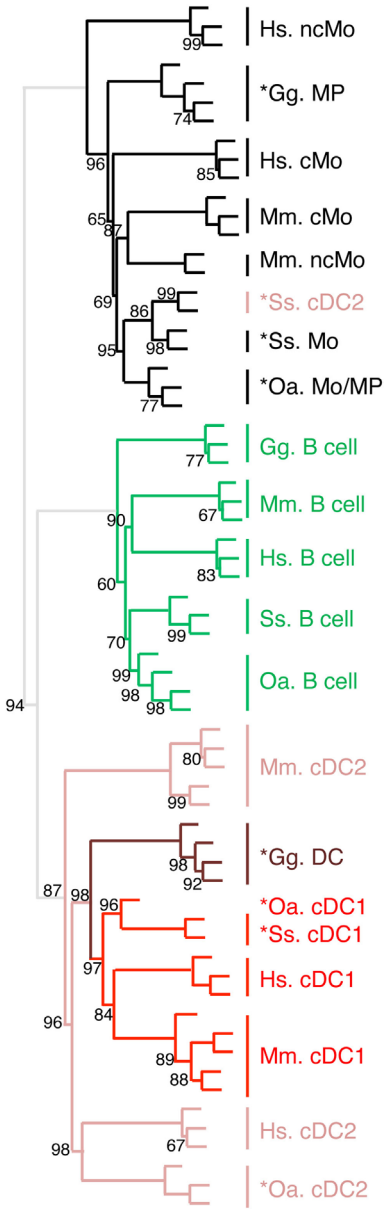

FIGURE 8 | Unsupervised cross-species hierarchical clustering including a chicken dataset demonstrates a conserved organization of vertebrate mononuclear phagocytes in the two main lineages of Mo/MP vs. cDC. An unsupervised cross-species hierarchical clustering analysis was performed as described in **Figure 5**, but including gene-expression data from chicken (Gg prefix for *Gallus gallus*) and focused only on the cell types commonly sorted in all five vertebrate species, i.e., B cells, Mo/MP, and cDC. The corresponding filtered dataset included 388 unique orthologous genes found regulated across cell subsets in each species.

RAD17 (53) or the *SP140* gene that encodes a nuclear body protein (54) (**Table 1**). Altogether, the results of the functional analysis of the conserved signature of B cells support the biological relevance of the conserved gene signatures generated by our approach.

In the conserved signatures corresponding to the DC lineage, the pDC signature is restricted to few genes including *RUNX2*, which encodes for a major known regulator of pDC development (56) and other genes whose role is not yet known in this subset, with three of them coding for potential cell surface markers or targeting molecules, i.e., the low density lipoprotein receptor-related protein 8 (*LRP8*), tetraspanin 13 (*TSPAN13*), and a zinc-family transporter protein member (*SLC30A5*) (**Table 1**). These genes, except *SLC30A5*, map to a common network (**Figure 9A**). No functional annotation was found significantly enriched in the pDC absolute signature due to the low number of associated genes. Interestingly, the pDC vs. cDC relative signature includes genes belonging to a regulatory network pointing to IFN −α/β production (**Figure 9B**) and retrieves as a major putative upstream regulator X-box binding protein 1 (*XBP1*) ($p = 10^{-15}$) (**Figure 11A**), a transcription factor involved in mouse DC development (57). The pDC vs. cDC relative signature was also enriched for "proliferation of B lymphocytes" ($p = 10^{-4}$), "morphology of B lymphocytes" ($p = 10^{-5}$), and "B cell receptor signaling" pathway ($p = 10^{-2.9}$), similarly to the conserved B cell signature (**Figure 11B**). These observations are consistent with the known usage downstream of mouse and human pDC endocytic receptors of a signaling pathway akin to that of the B cell receptor (58). This known pDC signaling pathway involves the products of *SYK*, *BLNK*, and *PIK3AP1*, three of the six genes responsible for the enrichment of the "B cell receptor signaling" pathway in the conserved pDC vs. cDC gene signature (Data Sheet S4 in Supplementary Material), as well as *CARD11* which contributes to the enrichment for the annotation "proliferation of B lymphocytes" in the pDC vs. cDC signature. This strongly suggests that this signaling pathway is conserved in pDC of all mammalian species. Beside *TCF4* which encodes for a major known regulator of both B and pDC development (52), several other genes associated to B cell biology are found in the pDC vs. cDC relative signature (**Table 1**), namely *CD79B*, *PTPRCAP*, *SEMA4D*, *CTCF*, *IFR1*, and *MEF2C*. This suggests that additional biological processes shared between B cells and pDC remain to be identified.

No absolute signature could be generated for cDC but interesting informations were obtained with relative signatures, i.e., the cDC vs. Mo/MP and cDC vs. pDC. The cDC vs. Mo/MP signature includes *FLT3*, a key gene in mouse DC development (59) as well as many genes of a regulatory network including *BCL11A*, *HLA-DOA*, *HLA-DRA*, *HLA-DMB*, *HLA-DOB*, *CD74*, the axone guidance neuron navigator *NAV1* and the MHC class 2 transcription regulator *RFX5* (**Figure 9C**). In relation to this network, *IL27* ($p = 10^{-4.4}$), *IFNG* ($p = 10^{-2.6}$), and NFkB ($10^{-2.8}$) were retrieved as putative upstream regulators (**Figure 11A**). The cDC vs. Mo/MP signature was enriched for canonical pathways such as "antigen presentation" ($p = 10^{-9.6}$), "DC maturation" ($p = 10^{-4.6}$), and "T helper cell differentiation" ($p = 10^{-6.2}$) (**Figure 11B**). The cDC vs. pDC signature includes a main regulatory network encompassing *PIK3CB*, *ICAM1*, *CLEC7A*, *HLA-DRA*, *IL1B*, and *LGALS3* (**Figure 9D**) and is enriched for "functions of antigen-presenting cells" ($p = 10^{-11.1}$), "inflammatory response" ($p = 10^{-8.9}$), "bacterial infection" ($p = 10^{-7.9}$), "migration of cells" ($p = 10^{-11.4}$), and "clathrin-mediated endocytosis signaling" pathway ($p = 10^{-4.6}$) (**Figure 11B**). TNF ($p = 10^{-9}$), RELA ($10^{-5.8}$), NFKB1 ($10^{-6.1}$),

TABLE 1 | Conserved gene signatures for mammalian mononuclear phagocytic cell subsets.

Cell subset gene signatures	genes conserved in 3/3 or 4/4 species[a]	genes conserved in 2/3 or 3/4 species[b]
B cell	*TRAF5; SP140; RAD17; MEF2C; MBD4;* ***FCRL1***[c,d,e]*;* ***CD19***	***VPREB3****; RFX5;* ***PAX5****; BACH2; AFF3; SWAP70; PLEKHA2;* ***MS4A1****; DMXL1;* ***CR2****;* ***CD79B****; CD22;* ***BLK****; ELL3; STRBP;* ***EBF1***
cDC vs Mo/MP	*NAV1; MSI2;* ***HLA-DMB****;* ***FLT3****; BCL11A*	*RFX5; PLEKHA5;* ***HLA-DOA****; BCAT2; AFF3; FAM149A; APOBEC3H; UVRAG; SPINT2; PDXP;* ***HLA-DOB****;* ***CD74****; CD5; AP1S3;* ***HLA-DRA***
cDC vs pDC	*WDR41; WDFY3; TPM4;* ***TLR2****; SPI1; SNX14; SNX10; SERPINB1;* ***SAMHD1****; RIN3; REL; RAB32; NHSL1; NCOR2; NAV1; MARCKS; LYZ; LGALS3; KLF3;* ***JAK2****; ITGA5; IL4I1;* ***IL1B****;* ***IFNGR1****;* ***IFI30****;* ***ID2****;* ***ICAM1****;* ***HLA-DMB****; GCA; FGL2; F11R; ETV3; DOCK7; DENND4A; CXCL16; CLEC7A; CHSY1;* ***BATF3****; ATP2B1; ARRB1; ARHGAP22;* ***ANPEP****; AIM1; AIF1; AHR; ADAM8*	*YWHAH; TPCN1; TDRD7; SNX21; SLC7A10; SIPA1L3; RGS12; MYO1D; MRC2; METRNL; MEA1; LRRK2; LRRC8C; LOXL3;* ***HLA-DQB2****; HAVCR2; FGF17; EHF; DOK1; DGKH; ATXN1; ASB2; ARHGAP26; ACTR3; RNF144B; PLEKHO2; MYOF; LPCAT2; KANK1; FAM114A1; DENND5A; ZNF524; VASP; SULT1A1; SPRED1; SNX8; SH3BP1; SH3BGRL;* ***RELB****; RALB; RAC1; PTPN12; PLEKHO1; PIK3CB; PAK1; NR4A1; NAB2; LFNG; JUNB;* ***IFNGR2****; IER2; HFE; FAM49B; EPSTI1; EGR1; EFHD2; DHRS3; CTBP2; COTL1;* ***CD74****; CD63; CBFB; C9ORF72; C1ORF21;* ***BCL6****; BASP1; ANXA5; SR140; PKM2;* ***HLA-DRA****; RGS4; TMSB4X; GMIP; MAST2; CXCL9; DNAJA4; KIF14; MTUS1; RABGGTA; RTN1; SYNJ1; TBX3*
DCs vs (Mo/MP & MoDC)	*MSI2; BCL11A*	*RAB34;* ***PDCD1LG2****; CHCHD7; CCL17; CARM1; AUH;* ***VEGFA****; UBA3; TUBA1A; TSKU; TMEM159; SLC48A1; SIGMAR1; RNF181; PTGR1;* ***NOS2****; IKBIP; FAM162A; BHLHE40*
MoDC	*TPI1; NDUFV2;* ***FCGR2B****; CD200R1; ALDOA*	
MoDC vs Mo/MP	*TPI1; SLC2A1; SLAMF1; PRNP; PPA2; POLR1D; PLAU; PALLD; NDUFV2; NARF; MRPL4; IL1R2;* ***FCGR2B****; EGLN3; DGKA; CSNK2B; CISH; CD200R1; AVPI1; ALDOA; ADAMTSL4*	*ZNF747; ZNF219; WIBG; VDR; SLC45A4; ROGDI; RASSF7; RAB34; RAB33A; PDE6D;* ***PDCD1LG2****; PBX2; NAGS; KCNK6;* ***ICOSLG****; HRH1; GOLGA8B; GOLGA8A; ETHE1; ERCC6; DVL2; DGUOK; CLEC10A; CHN2; CHCHD7;* ***CD209****; CCNG2; CCL17; CARM1; C1ORF122; AUH; ANKRD37; ZEB1;* ***VEGFA****; UBA3; TUBA1A; TSKU; TMEM159; TCTEX1D2; STRA13; SPATA24; SNRNP27; SLC48A1; SIGMAR1; S1PR3; RNF181; RMND1; RAB7A; PTGR1; PIGU; PI4K2A; OST4; NSL1;* ***NOS2****; NAE1; MT1A; MORN4; LMF2; JKAMP; IKBIP; IFT46; HAUS4; GLTPD1; GATC; FAM162A; FAM13A; FAM134A; ESYT1; ERI2; EEPD1; DNLZ; DHRS11; DCTPP1; CENPW; BHLHE40; APOO; AKIP1; CD1B; CGREF1; NOSTRIN; OLFM4;* ***GAS6****; SLC27A3*
(Mo/MP & MoDC) vs DCs	***CEBPB****; CCDC93;* ***C5AR1***	***TLR8****; FTL; DOK3;* ***CD68***
Mo/MP vs cDC	***TLR4****;* ***SOD2****; RBMS1;* ***LAMP2****; GLUL; FNDC3B; CYBB;* ***CEBPB****; CCPG1; CCDC93;* ***C5AR1***	***TLR8****; SNX27; RHOQ; OSTM1; KIF1B; FTL; DUSP6; DOK3;* ***CTSD****;* ***CTSB****;* ***CD68****; HERC5; IPMK; DPYD*
Mo/MP vs MoDC	*WDR33; VPS13D; UBE2D2; TRA2A; STAG2; SFPQ; NSD1;* ***NFKB1****; NADK; ITPR1; CFLAR; ARFGEF1*	*ZNF407; VPS13C; USP31; SLC16A4; SKAP2; PRKCH; PPFIA1; PIAS2; MDN1; MAP3K5; LRRC8D; CHM; AKAP13; ACTR3; SFRS2IP; RAD51L1; NAT12; MYST3; CDC2L5; ZNF830; ZBED5; TPPP3; TMEM164; TGS1; TBC1D8B; SNRNP35; SMEK1; SLC38A10; SHISA2; RSRC2; REV1; RALGAPB; PWWP2A; PRRC2B; PBRM1; NLRC5; MOGS; MAP7D1; LUC7L3; LIMCH1; KDM4C; ISY1; IP6K1; HNRNPUL2; HNRNPU; HNRNPK; HNRNPH2; HNRNPH1; HNRNPD; HNRNPA1; FOXN3; FAM173B; FAM159B; ERVW-1; CELF2; C9; NUP210L; PDZK1; ALMS1; LAMB1; METTL3; PAIP1*
pDC	***RUNX2***	*LRP8; INPP4A; TSPAN13; SLC30A5; GPM6B*
pDC vs cDC	*UBR2; UBE2H; TMED3;* ***TCF4****; TARBP1;* ***SYK****; STT3B; SPCS3; SNX5; SLC39A7; SIT1; SEMA4D; SEC61A1; SCYL3; SCAMP2; SAP30BP;* ***RUNX2****; RDH11; RASGRP2; RABAC1; PPAPDC1B; PGM3; PARN; PAG1; OGT; NUCB2; MSI2; MEF2C; LMAN2; IQCB1; IFT52; HBS1L; GPAM; GORASP2; FKBP2; FAM3C; EIF2AK3; DERL1; DDOST; DAD1; CYBB; COPA; CDC42SE2; CD4; CD164; BTRC;* ***BLNK****; BCL7A; ATP2A3; ATG5*	*ZXDC; VPS13A; UEVLD; TNRC6B; TMEM63A; TAF9B; TAF1A; SUSD1; STOML1; ST6GALNAC4; SSR2; SRPRB; SPG20; SLC38A6; SLC38A1; SLC25A36; SGCB; SERPINI1; SEC24C; SAP130; RAPGEF2; RALGPS1; RAB28; RAB11FIP2; PTAR1; PIK3AP1; OSTM1; NRP1; MYB; MGAT4A; MCOLN2; MCOLN1; LRP8; KIF13B; KIAA0226;* ***IRF7****; INPP4A; IMPACT; HIVEP1; FKBP8; FANCD2; FAM122B; DMTF1; CSTF1; CREB3L2; COBLL1; CBX4; CANX; ATG4D; ANKRD28; ANKIB1; AGBL3; AFF3; TPRG1L; RNF144A; IFI27L1; FAM65B; ELMOD3; DCAF7; CARS2; ZMYND11; YPEL3; USP24; TUBGCP6; TSPAN13; TRAM1; TOE1; TMEM138; TM9SF1; TCTA; SURF4; STAMBPL1; SSR3; SPCS2; SPATA13; SNX9; SLC7A5; SLC44A2; SLC30A5; SEPP1; SCAND1; SCAMP3; RHOH; RHBDF2; RHBDD1; REXO2; QDPR; PYCR2; PTPRCAP; PRMT7; POLD1; PEX5; NSUN3; MTMR9; LPGAT1; INTS7;* ***IFNAR1****; HM13; GRAP; GANAB; FNDC3A; FASTK; EXOC7; ELOF1; ELMOD2; CTCF; COPE; COMMD6; CNP; CIRBP; CDS2; CD79B;* ***CARD11****; C19ORF10; C16ORF80; C10ORF88; BTD; BET1; ARHGAP12; AHI1; WDR51B; SAPS3; MLF1IP; KIAA1370; CYBASC3; CEP110; CCDC111; ANUBL1; MME; PTPRS; ATF2; GPM6B; MON2; PPM1A; TM7SF3; TMCO1; UGCG; ZDHHC14; ZNF521; TMED10; PAIP1*
cDC2		*FCER1A*
cDC1	***XCR1****; WDFY4; FNBP1;* ***FLT3****;* ***CADM1***	*SNX22; GCET2*
cDC2 vs (pDC & cDC1)	*TRPS1; STK24; SLC16A3;* ***SIRPA****; SIGLEC8; S100A4; RIN2;* ***REL****;* ***PILRA****; NFAM1; NCF2; MAFB; LRP1; ITGAM;* ***IL1R2****;* ***IL1B****; IGSF6;* ***IFI30****; FHL3; EPB41L3; DOCK4; DHRS3; CSF3R;* ***CSF1R****; CLEC4A;* ***CD300A****; C19ORF59; ADRBK2; TREM1*	*TNFRSF1B;* ***TLR8****; TICAM2; STK10; SP2; SLFN12; SIGLEC9; SIGLEC7; RNASE2; PHF21A; LST1; LIMD2;* ***LILRB2****;* ***LILRB1****;* ***LILRA6****;* ***LILRA3****; IFITM2; GNGT2; GBP4; FAM111A; EMR1; DPP10; DENND1A;* ***DDX58****; CDKN2B; CD300LF; CD300LB;* ***CD209****; C10ORF11; ADAP1; CLEC6A; DAGLB; WDR45L; SIGLEC5; SFRS5; S100A12; PLEC1; MYST1;* ***MX2****; MS4A8B; LRRC33; HSPA6; GK3P; GAPDH; FAM45B; CEBPD;* ***CD1E****; CD1B; FCER1A; KSR1;* ***OAS2****; PTGER3*

(*Continued*)

TABLE 1 | Continued

Cell subset gene signatures	genes conserved in 3/3 or 4/4 species[a]	genes conserved in 2/3 or 3/4 species[b]
cDC2 vs (pDC & cDC2)	***XCR1***; *WDFY4; ST3GAL5; RAB32; PPT1; PPA1; LRRC1; KIAA1598; FNBP1;* ***FLT3***; *CALM1;* ***CADM1***	*SNX22; PPAP2A; PLEKHA5; GRAMD2; DENND1B; CLEC1A; ATXN1; FAM114A1; HEPACAM2; PI4K2A; PLEKHO2; WDR91; TRIO; RALB;* PKP4; *PDLIM7; G3BP2;* ***BCL6***; *ATPIF1; GCET2; BRWD2; FGD6; MYO9A*
ncMo vs cMo[f]	*ACAT2; ACE; ACOT9; ADRBK2; ANKRD42; APOA2; ASB2; BDKRB2; BGLAP; C1ORF112; C1ORF56; C20ORF112; CAPZB; CBX4;* CD4; CD83; CDH24; *CHD5;* CSF1R; *CYP2R1; DCBLD1; DDB2; DDIT4; DLGAP4; FBP1; GABBR1; GLMN; GNE; GNPNAT1; GPT; GRHPR; HEY1; HN1; IL12RB1; IL17A;* IL2RG; KCNMA1; *KCTD11; KNDC1; LMX1B; LUZP1; MAFF; MPZL1; MUTYH; MYOD1; NCAPH2; NCOR2; NFKBIA; NPAS2; NUB1; PCK1; PDCD4; PGR; PITPNM1; PLEKHH1; PMF1; PMVK; POLR3H; RAB25; RAD52; RFC5; RHOF; RSAD1; RWDD3; SECISBP2; SERPINA1; SH2D3C; SIRT5; SLC37A1; SMS; ST3GAL1; ST3GAL5; TBC1D8; TCF7L2; TNNC1; U2AF1L4; UNG; WDR76*	
cMo vs ncMo[f]	*AACS; ABHD5; AGTPBP1; ALDH2;* ALOX5AP; *ANXA1; AOAH; ARL8B; ATP6V1A; ATP6V1B2; ATP6V1C1; AUH; B4GALT1; C19ORF59; C5ORF15; CCR1; CD164; CD84; CETN2; CLTA; COPB2; CSF3R; CYP27A1; DCLRE1A; DNAJC10; ECE1; EHD4;* ***EIF2AK2***; *EIF2AK3; ENSA; ENTPD7; ERP29; EXOC5; F13A1;* F5; *FAM102B; FAM63A; FBXL5; FBXO9; FN1; GBE1;* GNA12; *GNPAT; GSN; GYS1; HMGB2;* ***IL1R2***; ***IL1RN***; *ITM2B; KEAP1; LACTB; LCN2; LEO1; LMAN1; LMNB1;* ***LYZ***; *MBD5; MBIP; MGA; MPP1; NHLRC2; NISCH; NKRF; NPC1; NSF; NUCB2;* PAM; *PARP8; PDE2A; PGD; PLCB1; PNPLA8; PON2; PREPL; PRKAR1A; PRUNE; PSMA1; PSTPIP1; PUM2; PXK; PYGL; RAB27A; RAB3D; RABGAP1L; RARS; RHOT1; RMI1; RNF130; RPGR; RSC1A1;* ***S100A8***; *SCRN3; SCYL1;* SDCBP; *SEC22C;* SELL; *SENP5; SERPINB1; SHB; SIGLEC1; SLC16A7; SLC25A44; SLC35B3; SLC39A9; ST8SIA4; TBC1D2; TEX2; TGM1; TM6SF1; TMEM161B; TMEM71; TPCN1; TREML2; TRIP11; TSHZ1; UBE4A; UMPS; USP10; UXS1; VAPB; VNN3; VPS37B; WDTC1; XBP1; ZMYM4*	

[a] *Genes conserved in 4/4 species, or in 3/3 species for cDC2, cMo, and ncMo since only three species could contribute to the analysis.*

[b] *Genes conserved in 3/4 species or 2/3 species.*

[c] *Genes in bold were previously demonstrated to play a significant role in the development or functions of the population of interest.*

[d] *Underlined genes were annotated as located in "plasma membrane" according to Ingenuity Pathway Analysis.*

[e] *Genes highlighted in gray have been previously identified as signatures genes for the corresponding mouse and human cell populations in our earlier study (15).*

[f] *Signature genes of the relative cMo vs. ncMo and of the ncMo vs. cMo signatures were provided only for the 3/3 species selection since the gene lists for the 2/3 species selection encompassed hundreds of genes.*

P38 MAPK ($10^{-5.5}$), IFNG ($10^{-5.4}$), and to a lesser extent STAT3 ($10^{-4.7}$) and CSF2 ($10^{-4.3}$) are predicted as putative upstream regulators in this signature (**Figure 11A**). In addition, this cDC vs. pDC signature includes *BATF3*, a gene highly expressed in cDC that is key in cDC1 development in mouse and human (60), as well as *ARHGAP22*, a gene involved in actin cytoskeleton regulation (61), that was initially described as a top gene of the absolute cDC signature common to human and mouse (15). Altogether, the relative gene signatures of cDC emphasize their nature of highly endocytic, motile, and expert antigen-presenting cells throughout species.

The conserved cDC1 signature encompasses genes with known contribution in the biology of this lineage, such as *XCR1*, *FLT3*, and *CADM1* (59), as well as additional genes which biological function in this subset remains enigmatic, such as the germinal center B-cell-expressed transcript 2 protein (*GCET2*),

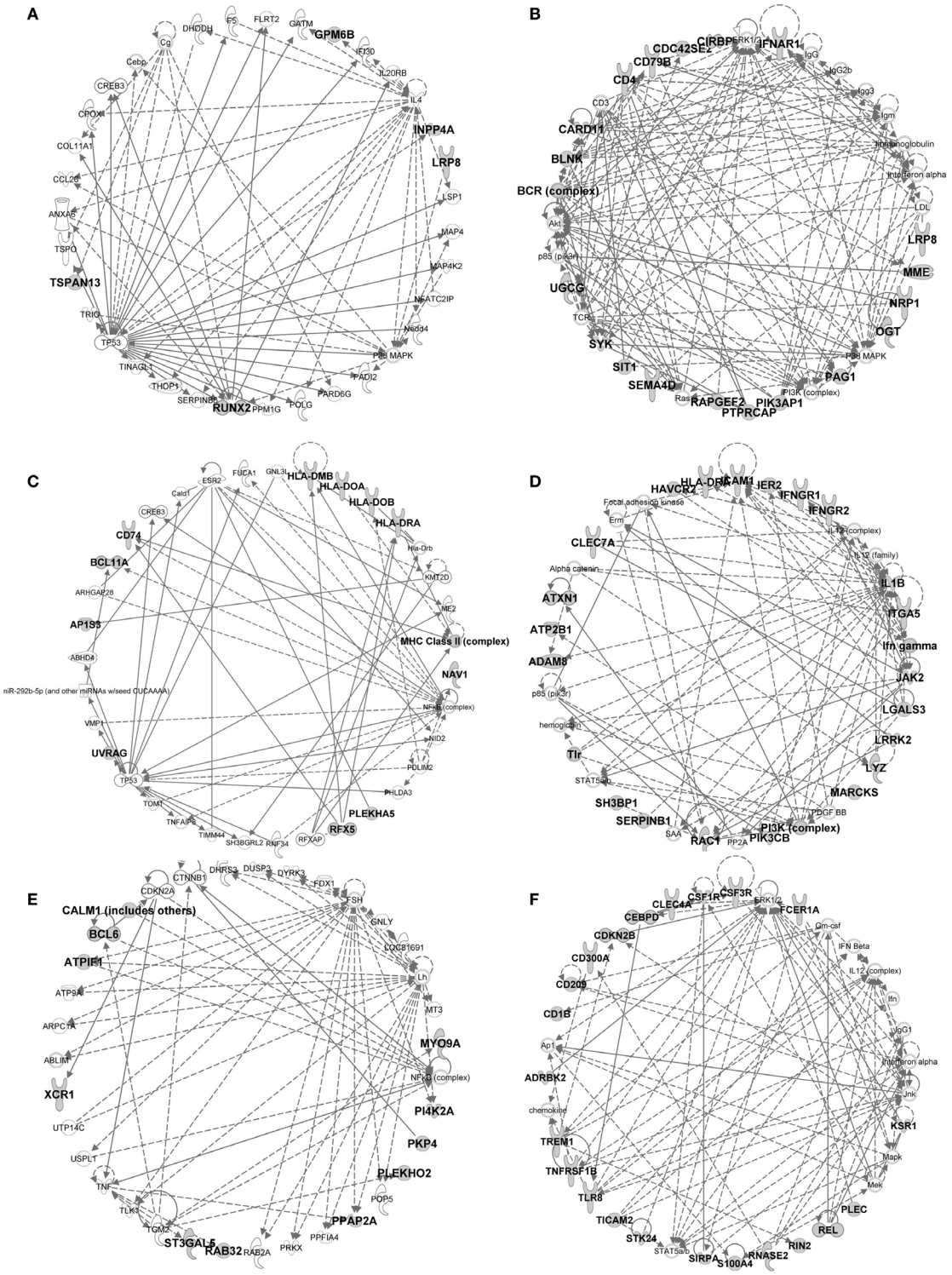

FIGURE 9 | IPA gene interaction networks of the conserved signatures in subsets of the DC lineage. The conserved signatures of subsets of the DC lineage were analyzed in Ingenuity Pathway Analysis which generates networks based on the connectivity of the genes in each signature (in boldface) but also on their connectivity with genes not belonging to the signature (in plain characters). The identified networks are displayed as graphs showing the molecular relationships between genes/gene products. Genes are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). The edges can represent direct (continuous) or indirect (dashed) relationships between nodes. Selected networks generated by IPA and covering parts of conserved cell-specific signatures are displayed: **(A)** pDC signature network, **(B)** pDC vs. cDC signature network, **(C)** cDC vs. Mo/MP signature network, **(D)** cDC vs. pDC signature network, **(E)** cDC1 vs. (pDC and cDC2) signature network, **(F)** cDC2 vs. (pDC and cDC1) signature network.

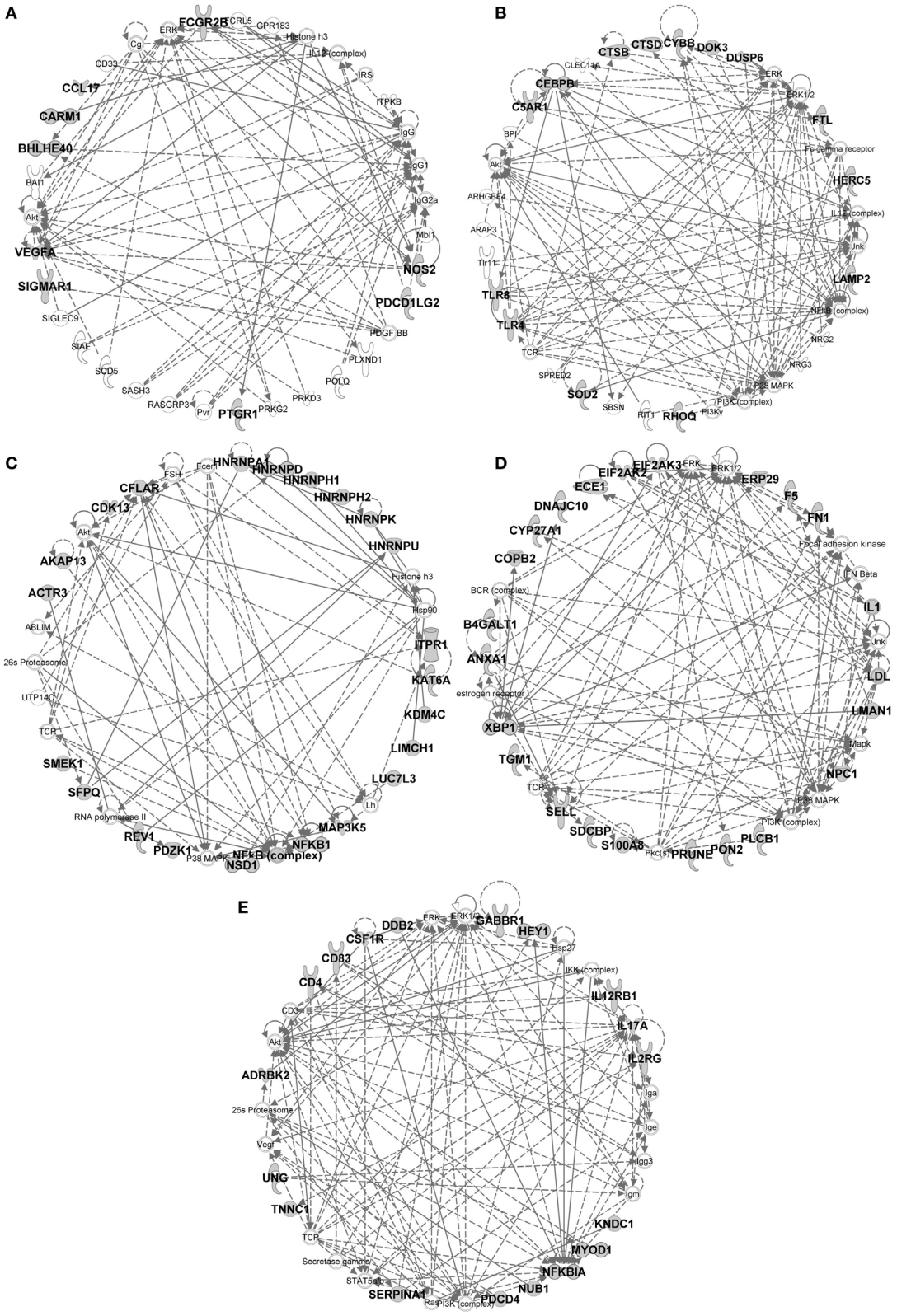

FIGURE 10 | **IPA gene interaction networks of the conserved signatures in subsets of the monocytic lineage**. The conserved signature of subsets of the monocytic lineage were analyzed in Ingenuity Pathway Analysis as in **Figure 9**. The selected networks displayed are: **(A)** MoDC signature network, **(B)** Mo/MP vs. cDC signature network, **(C)** Mo/MP vs. MoDC signature network, **(D)** cMo vs. ncMo signature network, **(E)** ncMo vs. cMo signature network.

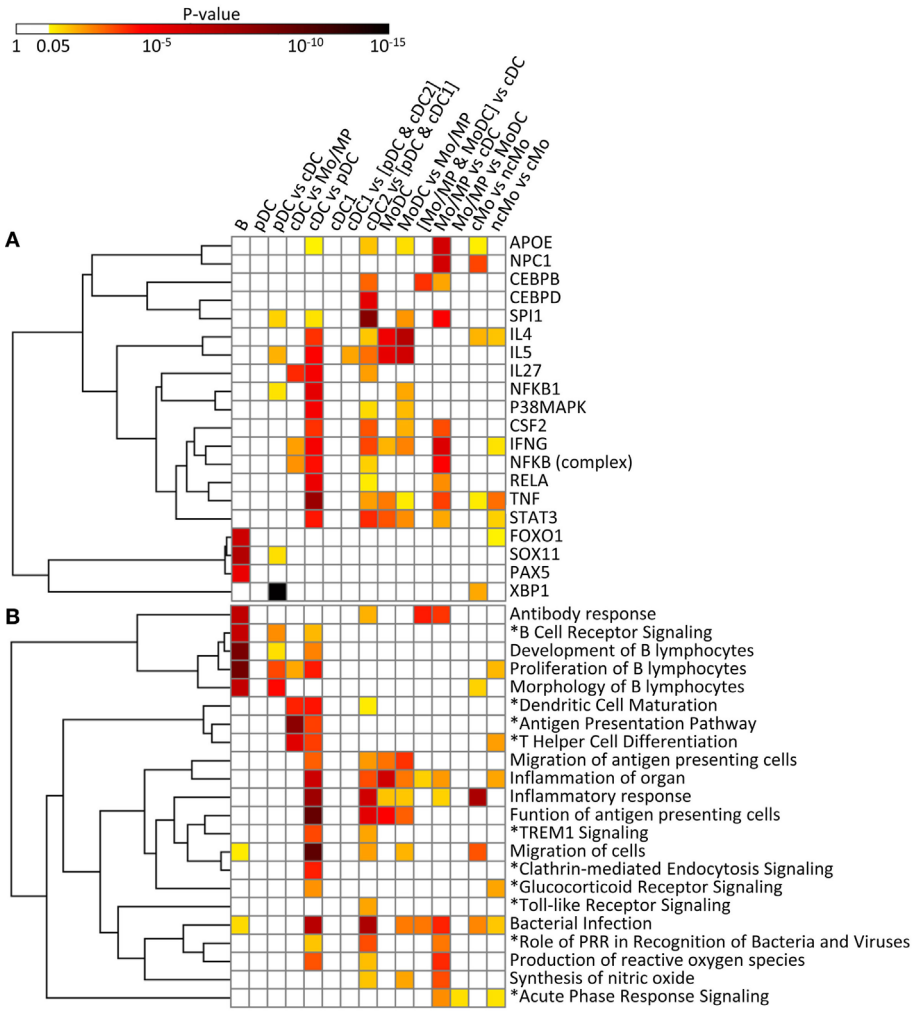

FIGURE 11 | IPA analysis of the conserved cell type gene signatures: upstream regulators (A) and biological functions and canonical pathways (B). A promoter sequence analysis of the conserved cell type gene signatures performed using IPA is displayed as a heatmap of the *p*-value [upstream regulators, **(A)**]. A biological function and canonical pathway analysis of the conserved cell type gene signatures performed using IPA is displayed as a heatmap of the *p*-value **(B)**. Selected upstream regulators and functions and pathways (*), in **(A,B)**, respectively, were classified using hierarchical clustering based on the average linkage metrics. Enrichments were considered significant when supported by at least three genes and by a *p*-value ≤0.05.

the WDFY family member 4 (*WDFY4*) whose polymorphism is associated to autoimmune diseases (62), and two intracellular trafficking proteins, a formin-binding protein (*FNBP1*) (63) and Sorting Nexin-22 (*SNX22*) (64) (**Table 1**). The conserved cDC1 vs. (pDC and cDC2) relative signature provides a longer list of genes belonging to an interaction network that includes *BCL6*, a transcriptional repressor that was recently found involved in the specification of cDC1 (17) as well as *XCR1* and *CALM1* (**Figure 9E**). IPA did not retrieve significant annotations for the cDC1 absolute or relative gene signatures. This emphasizes how little is currently known on the molecular regulation of the functions specific to cDC1s, such as cross-presentation. Future studies investigating in mouse cDC1s the functional role of the genes identified here as being part of the conserved cDC1 signatures will advance our understanding of the functions of these cells and their molecular regulation.

The absolute cDC2 conserved signature was empty. Many genes of the relative cDC2 vs. (pDC and cDC1) signature belong to a network that includes *SIRPα* (*CD172A*), a selective marker of cDC2 within the DC lineage (44), together with *CSFR1*, *TREM1*, *CLEC4A* (also known as *DCIR*), *CD1B*, and *RELB* which is known to control mouse cDC2 differentiation (65) (**Figure 9F**). A second network includes *ITGAM* (*CD11b*), a marker used to identify mouse cDC2, *CLEC6A*, and *IL1B* (Figure 7 in Supplementary Material). SPI1 ($p = 10^{-9.8}$), CEBPD ($10^{-6.2}$), and CEBPB ($10^{-3.6}$) are predicted upstream regulators, as well as CSF2 ($10^{-3.8}$), STAT3 ($10^{-4.4}$), and IFNG (10^{-4}) which were already enriched in the cDC vs. pDC signature (**Figure 11A**). This cDC2 relative signature also includes *IFI30*, also known as *GILT*, a lysosomal thiol reductase important in MHC class II and class I antigen processing (66, 67) (**Table 1**). This relative signature is enriched for "function of

antigen-presenting cells" ($p = 10^{-6.2}$), "inflammatory response" ($p = 10^{-6.9}$), and for the pathways "TREM1 Signaling" ($10^{-2.5}$), "Toll-like receptor canonical signaling" ($10^{-2.5}$), and "role of pattern recognition receptors in recognition of bacteria and viruses" ($p = 10^{-3.9}$) (**Figure 11B**). Other genes were uncovered that may be important regulators of the function of cDC2s or which product could be used to identify or target these cells, including the genes coding for plasma membrane proteins such as glycoprotein *CD300A*, the sialic binding lectin *SIGLEC8*, and the paired immunoglobin-like type 2 receptor *PILRA*. This conserved relative signature shows that within the DC lineage throughout species, cDC2 express specific networks of genes related to pathogen sensing, antigen presentation, IL-1β production, and inflammation.

In the conserved signatures corresponding to the monocytic lineage, the absolute MoDC and relative MoDC vs. Mo/MP signatures are enriched for "inflammation of organ" ($p = 10^{-6.9}$ and $p = 10^{-3.3}$), "function of antigen-presenting cells" ($p = 10^{-5.1}$ and $p = 10^{-3.6}$), and "migration of antigen-presenting cells" ($p = 10^{-3.3}$ and $p = 10^{-4.3}$) (**Figure 11B**) and encompasses *NOS2*, *CCL17*, *VEGFA*, and *FCGR2B* that map to a common major network (**Figure 10A**). IL-4 ($p = 10^{-5.7}$) and IL5 ($p = 10^{-5.9}$) are predicted regulators (**Figure 11A**). Among other genes of interest that had not yet been associated to MoDC are the triose phosphate isomerase *TPI1*, the NADH dehydrogenase flavoprotein *NDUFV2*, the aldolase *ALDOA*, and the *CD200R1* gene that encodes for an inhibitory cell surface receptor of MP functions (68) (**Table 1**). The relative MoDC vs. Mo/MP signature encompasses additional genes that participate in "migration of cells" ($p = 10^{-2.3}$, with *S1PR3*, *CCL17*, and *SLC2A1*), "bacterial infection" ($p = 10^{-3.2}$, with *CD1B*, *CD209*, and *FCGR2B*), and "synthesis of nitric oxide" ($10^{-2.5}$, with *PLAU*, *IL1R2*, and *NOS2*) (**Figure 11B** and Data Sheet S4 in Supplementary Material). The conserved MoDC signatures indicate a dominant association of this subset to inflammation, as well as to DC functional properties when compared to Mo/MP across species.

Most of the genes in the Mo/MP vs. cDC conserved signature had been previously identified as overexpressed in murine MP, such as *TLR4*, *CEBPB*, *C5AR1*, and *SOD2* (9, 15) (**Table 1** and **Figure 10B**). A significant proportion of the genes within this signature are related to "inflammation of organ" ($p = 10^{-2.7}$), "production of reactive oxygen species" ($p = 10^{-4.4}$), "synthesis of nitric oxide" ($p = 10^{-3.9}$), "bacterial infection" ($p = 10^{-4.6}$), "role of pattern recognition receptor in recognition of bacteria and viruses" ($p = 10^{-3.3}$), and "acute phase response signaling" ($p = 10^{-2.9}$) (**Figure 11B**). Putative upstream regulators are NPC1 ($p = 10^{-6.8}$), APOE ($p = 10^{-6.8}$), IFNG ($p = 10^{-6.4}$), SPI1 ($p = 10^{-5.2}$), and NFkB ($p = 10^{-5.1}$) (**Figure 11A**). Additional proteins are potential transcriptional regulators of importance in Mo/MP, such as the RNA-binding protein RBMS1 and the cell cycle progression factor CCPG1. The Mo/MP vs. MoDC signature includes a gene network centered on *NFkB* and *MAP3K5* (**Figure 10C**). Overall, the conserved Mo/MP relative signatures support the association of Mo/MP to inflammation and oxidative stress across species.

The conserved comparative signature of cMo vs. ncMo retrieved genes belonging to a network with *IL1*, fibronectin (*FN*), *S100A8*, and *XBP1* (**Figure 10D**), the latter being proposed as an upstream regulator ($10^{-2.5}$) together with *NPC1* ($p = 10^{-4}$) (**Figure 11A**), and is strongly associated to "inflammatory response" ($p = 10^{-8.4}$) (**Figure 11B**). The reciprocal ncMo vs. cMo signature includes a gene network with *IL17A*, *CSFR1*, *NFKBIA*, and *serpinA1* (**Figure 10E**), and is significantly associated to the "glucocorticoid receptor signaling pathway" ($p = 10^{-2.5}$) and to some extent to the "inflammation of organ" ($p = 10^{-2.6}$) (**Figure 11B**). These relative signatures indicate that cMo have a conserved gene program directed to strong inflammation, whereas ncMo, a poorly understood subset, might be exquisitely regulated by glucocorticoids as suggested in the literature (69, 70).

Altogether, the mononuclear phagocyte system from distantly related mammals is composed of a diversity of subsets that belong to the DC or to the Mo/MP lineage and express discriminating gene signatures involved in distinct regulatory networks and biological functions conserved through mammalian evolution. In most instances, the subset signatures also point to several unexpected genes and upstream regulators that are likely to be important in the subset biology since their selective expression pattern across subsets of mononuclear cells is conserved across species.

Phylogenetic Evidences for the Existence of a Gene Repertoire for Mononuclear Phagocyte Subsets in Birds and Bony Fishes

The existence of orthologous genes of the conserved mononuclear phagocyte subset signatures in reptile/birds, fishes, and agnathans, would indicate that the genetic equipment for mononuclear phagocyte subset diversity is available in vertebrate species distant from mammals. In the case of birds and reptiles, it remains unknown whether they have pDC, cDC1, and cDC2 subsets homologous to mammals. An orthology analysis of selected genes from conserved subset signatures revealed that most genes possess a unique ortholog in birds and reptiles, with conserved synteny with human, for instance *XCR1*, *BATF3*, *RUNX2*, *TSPAN13*, and *CSF1R* (**Table 2**). Furthermore, these same genes also possess one or more orthologs in fish. Multiple orthologs in fish are often due to the whole genome duplication that occurred during the evolution of teleosts, and to further local duplications. Importantly, fish co-orthologs of mononuclear phagocyte subset genes are generally supported by conserved synteny. Genes duplicated in fish may have been subjected to sub-functionalization, as it is the case for many immune genes duplicated in this group of vertebrates; however, some markers have a unique counterpart in fish genomes (like *BATF3*, *RFX5*, and *CIITA*), with copy loss possibly due to detrimental effects of duplication. The case of *MHC class II* is particular: although fish *MHC class II* genes are not always considered as true orthologs of human *MHC class II* genes, their sequences show the hallmarks of *bona fide* class II antigen-presenting receptors and they likely have similar functions. For c-type lectin-like (*CLEC*) molecules, no true orthologs can be identified in fish nor in birds/reptiles, as each branch of vertebrates – even each group of mammals – shows its own set of expanded *CLEC* genes. Altogether these data show that a repertoire of conserved genes for mononuclear phagocyte subsets exists in bony fishes and reptiles, which constitutes a list of candidates for relevant markers.

TABLE 2 | Search for the existence of orthologs in reptile/birds, fishes, and agnathans for selected genes of the conserved mononuclear phagocyte subset signatures.

		Reptiles/birds		Fishes		Agnathans (lamprey)	
Cell subset gene signatures	**Gene**	**Orthologs**	**Conserved synteny**	**Orthologs**	**Conserved synteny**	**Orthologs**	**Conserved synteny**
cDC vs. Mo/MP (MHC-related molecules)	*HLA-DR*	–	–	–	–	–	–
	HLA-DM	?	–	–	–	–	–
	HLA-DO	–	–	–	–	–	–
	CD74	+ (1)	Yes	+ (Multiple)	Yes	–	–
	CIITA	+ (1)	Yes	+ (1)	Yes	–	–
cDC vs. Mo/MP	*NAV1*	+ (1)	Yes	+ (Multiple)	Yes	–	–
	RFX5	+ (1)	Yes	+ (1)	Yes	–	–
	BCL11A	+ (1)	Yes	+ (Multiple)	Yes	–	–
MoMP vs. cDC	*CEBPB*	+ (1)	Yes	+ (1)	Yes	–	–
	C5AR1	+ (1)[a]	Yes[b]	+ (1)[c]		–	–
	SOD2	+ (1)	Yes	+ (1)	Yes	+ (1)	?
	APOE	–	–	+ (Multiple)	Yes	–	–
	TLR4	+ (1)	Yes	+[d]	Unclear	–	–
cDC1	*XCR1*	+ (1)	Yes	+ (Multiple)	Yes[e]	–	–
	FLT3	+ (1)	Yes	+ (1)	Yes	–	–
cDC vs. pDC	*BATF3*	+ (1)	Yes	+ (1)	Yes	–	–
	ARHGAP22	+ (1)	Yes	+ (Multiple)	Yes	+ (1)	?
	CLEC7A	–	–	–[f]	–	–	–
B cells	*CD79B*	+ (1)	Yes	+ (1)	Loose[g]	–	–
	PAX5	+ (1)	Yes	+ (1)	Yes	(+)[h]	?
	CD19	–	–	–	–	–	–
pDC	*RUNX2*	+ (1)	Yes	+ (1)[i]	Yes	+ (1)	?
	TSPAN13	+ (1)	Yes	+ (Multiple)	Yes	+ (2)	? (for both)
cDC2 vs. (cDC1 and pDC)	*IFI30/GILT*	+ (1)	Yes	+ (Multiple)	Yes	+ (1)	?
	CSF1R	+ (1)	Yes	+ (Multiple)	Yes	–[j]	–
	SIRPA	?[k]	–	–	–	–	–
	TREM1	–[l]	–	–	–	–	–
	CLEC4A	–	–	–[f]	–	–	–
	CLEC6A	–	–	–[f]	–	–	–
More or less cDC1-specific	*CLEC9A*	–	–	–[f]	–	–	–
	CLNK	+ (Turkey)	Yes	+ (1)	Yes	+ (1)[m]	?

[a]*Birds have one co-ortholog of human C5RA1 and C5RA2;* [b]*only in the lizard Anolis, not in available bird genomes;* [c]*fish generally have one co-ortholog of human C5RA1 and C5RA2;* [d]*only in some species: zebrafish, catfish, and salmonids;* [e]*see Ref. (36);* [f]*for all CLEC, no true ortholog, each deep branch of vertebrates has its own set of expanded CLEC;* [g]*the neighborhood is not conserved but zebrafish CD79B is close to Arhgap27 and Plekhm1 that are on the same human chromosome (chr17) as CD79B but at 20 megabases; CD79B genes often are not annotated in fish genomes. In zebrafish, CD79B is ENSDARG00000088902;* [h]*a lamprey gene ortholog to PAX5 has been identified and was selectively expressed in lamprey VLRB+ cells which resemble B lymphocytes (55); however, this gene is not identified in the current publicly available assembly of the lamprey genome;* [i]*duplicated in zebrafish and cavefish;* [j]*a lamprey gene is a co-ortholog to all vertebrate CSF1R, PDGFR, KIT, FLT3, etc.;* [k]*in birds species, several genes are co-orthologs of all mammalian SIRPs including SIRPA;* [l]*bird TREM-like genes are more closely related to TREM2 rather than to TREM1;* [m]*co-ortholog of CLNK, BLNK, and other related genes.*

The presence of *BATF3* and *XCR1* are hints at possible existence of cDC1 in these species, as *BATF3* specifically controls cDC1 development in mice (71) and *XCR1* expression is strictly associated to cDC1 in several mammals (34, 36, 40, 41). In contrast, the lamprey does not have identified orthologs for many of the genes selected from the transcriptomic fingerprints of the subsets of mammalian mononuclear phagocytes (**Table 2**). Agnathans, including lampreys and myxines, harbor three adaptive immune cell types, each expressing a specific class of variable lymphocyte receptors, VLRC, VLRA, and VLRB, and showing transcriptomic and functional commonalities with gnathostome γδ T lymphocytes, αβ T lymphocytes, and B lymphocytes, respectively (55, 72). However, it is uncertain whether or not the activation of agnathan lymphocytes requires APCs, and if so, to which extent these cells could resemble gnathostome APCs (72). Contrary to the situation in birds and fishes, our observations do not support the existence in the lamprey of gene sets similar to those defining the transcriptomic fingerprints of the mononuclear phagocytes of mammals. Although incomplete assembly and annotation of the genome of the lamprey do not allow drawing definitive conclusions, our observations are consistent with the lack in agnathans of MHC functional homologs and of the particular proteasome machinery used by mammalian APCs for antigen processing (72). Altogether, this phylogenetic study shows that the repertoire of key genes characterizing the diversity of the mononuclear phagocytes in mammals were already present in the common ancestor of tetrapods and fishes but might be largely absent in agnathans.

Discussion

Our computational transcriptomic meta-analysis indicates that the complex organization of the mononuclear phagocyte system shows conservation throughout distantly related mammals, a finding that appears to extend to chicken, a non-mammalian vertebrate. In the present work, by using GSEA and hierarchical

clustering for unbiased pan-genomic analysis of the molecular identity of immune cell subsets across four vertebrate species, we convincingly established the existence of strong homologies between these cell types across mammals, beyond the already known existence of B cells in all species. Specifically, we could align across mammals cDC1, cDC2, pDC, MoDC, Mo/MP, and cMo vs. ncMo. In addition, we found that many of the genes that we showed to be selectively expressed in distinct mononuclear phagocyte subsets in mammals have existing orthologs in bony fishes while this appears not to be the case in lamprey. Thus, our study suggests that conserved mononuclear phagocyte subsets might exist in all gnathostomes but not in agnathans. However, this hypothesis will require to be tested experimentally, by re-examining the presence of orthologous genes in lamprey upon completion of the genome assembly and its annotation, by identifying and studying candidate mononuclear phagocyte subsets in bony fishes, and by determining whether similar cells exist in sharks, rays, and lamprey. For example, orthologous genes of the conserved mononuclear phagocyte signatures (**Table 2**) could be targeted by the CRISPR/Cas9 technology with a reporter gene marker in order to identify and characterize mononuclear phagocyte subsets in bony fishes (73), with for certain genes the need to test several putative orthologs in fish due to genome duplication.

The two methodologies that we used to assess subset homologies across species, i.e., hierarchical clustering and GSEA, display complementary functionalities. Hierarchical clustering on filtered, centered, reduced, and aggregated datasets has the advantage of integrating all samples together into a single analysis and of providing a global overview of the homologies between cell subsets of various species (15, 17, 18, 74, 75). However, the integration of distinct datasets requires a cross-normalization procedure which consists in a rather profound mathematical transformation of the data. The normalization procedure artificially increases the variance for genes with only small differences in their initial signal intensities between the different cell types studied. Conversely, it comparatively decreases the variance for genes with high differences in their initial signal intensities between the different cell types studied. To limit the biases that this normalization introduces, it is thus necessary to select only the orthologous genes that vary strongly in their expression across the cell types examined within each species. Another corollary is that this analysis can only be applied to genes that have known orthologs in all species. If one ortholog is missing in only one species, the gene must be removed from the analysis. Hence, this method should be used with caution, only under conditions where dataset normalization does not yield too strong biases in gene-expression profiles. It is also not appropriate when the structures of the different datasets are too different (i.e., the number and potential identities of cell types vary too much across datasets), because the dynamic ranges of gene expression between datasets are not expected to be the same and should therefore not be forced to similarity. Even under conditions where the experimental design is favorable to the use of hierarchical clustering, GSEA ensures of the robustness of interpretation. GSEA has been used by us and others to perform cross-species comparisons (5, 19, 29, 42, 76–78). GSEA notably displays advantages and drawbacks distinct from those of hierarchical clustering. First, it is easier to perform GSEA since dedicated ready-to-use stand-alone programs are available which do not require bio-informatics expertise. Second, GSEA is more sensitive, notably to detect overlaps of common functions/gene networks between cell populations or cellular contaminations, as exemplified with sheep *pDC enriched in human and mouse B cell fingerprints. This higher sensitivity is linked to (i) the fact that GSEA can detect coordinate regulation of gene modules (geneset-based approach) and thus does not rely on the strong regulation of few single genes (single gene-based approach), (ii) the fact that GSEA, when applied to multiple species, takes into account all genes that have orthologous counterparts in the considered species and is not restricted only to highly variable genes. Third, GSEA can perform cross-platform comparison without any cross-normalization thus without any supplementary artificial manipulation of the expression data. Finally, it can be performed on multiple datasets, even if their structures are different. However, GSEA presents the limitation of performing pairwise comparisons whose results can be integrated and visualized with our Bubble GUM software, but it nevertheless does not provide a global trans-species overview of subset homology. Overall, in order to increase confidence in the interpretation of the results, it is important to combine both approaches and verify that they both lead to consistent conclusions.

Our subset assignment methodology demonstrates similarity or proximity between subsets across species but not strict identity. Besides possible intrinsic transcriptomic differences between species, one of the reasons that explain this limitation is the process of subset identification itself, which makes use of different surface markers. Whenever possible, similar marker combinations were used such as CADM1 and CD172 that are known to be conserved markers across human, mouse, and sheep cDC subsets (42). However, mAb anti-CD11c did not exist for the initial gating in pig and the mAbs in the exclusion pool were not the same in pig and sheep. Moreover, existing marker combinations are not always specific and can lead to cross-contamination between different cell subsets. Indeed, the GSEA of the sheep *cDC2 revealed that they may have been contaminated by pDC, despite our attempt to avoid this problem through exclusion of CD45RB-expressing cells. It remains possible that pDC expressing minimal levels of CD45RB were still present in the sorted *cDC2 population, and not in the sheep cDC1 subset. However, since sheep *cDC2 were found in the correct cDC branch of the hierarchical clustering, their contamination by pDC is likely to have been limited. Similarly, it is likely that the sorted sheep *pDC include residual B cells, explaining the enrichment for the human B cell fingerprint at a level above expectation: indeed after exclusion of B cells with a pan-B cell marker, sheep *pDC were selected with a mAb directed to CD45RB, which may react with residual B cells that have escaped the pan-B cell exclusion. Yet, sheep *pDC still cluster with other species pDC, separately from B cells. In the case of pig, pDC were selected using markers not expressed by B cells and they displayed an enrichment for B cell fingerprints at a level encountered in GSEA analyses of mouse pDC (Figure 4 in Supplementary Material). Finally, our approach was able to

demonstrate that *a priori* assignment of subset identity based on the expression of a few membrane markers could be wrong, like in the case of the pig *cDC2. Moreover, our approach had the power to properly re-assign cell subset identity, demonstrating that pig *cDC2 were actually homologous to mouse and human ncMo. Another laboratory analyzed the transcriptome of similar pig cells sorted as $CD14^{low}$ $CD163^{high}$ cells, but they could not assign them to classical nor to non-classical human Mo, due to differences in bio-informatics approaches in this study (79) and in ours.

Our study will help improving in the near future the toolbox available in each species for rigorous and consistent phenotypic identification of cell subsets, thanks to our identification of novel, conserved, and specific, combinations of surface markers for each cell subset, which should allow generating more appropriate staining reagents. For instance, fluorescently labeled recombinant XCL1 could theoretically be used in any species to rigorously identify and sort cDC1 (38, 41). In addition, cell surface proteins encoded by genes shown here to be selectively expressed in a conserved manner in specific subsets of mononuclear phagocytes represent new candidate markers to refine and homogenize phenotypic identification of these cells across species, such as LRP8, TSPAN13, NRP1, and SLC30A5 for pDC, FCGR2B, and CD200R1 for MoDC, SIGLEC8 and IGSF6 for cDC2, and CSF1R, TLR4, and C5AR1 for Mo/MP (**Table 3**). However, these potential new markers for subset identification need to be validated at the protein level.

The subset-specific signatures that are conserved throughout distant mammals included variable number of genes that were sometimes far lower than the numbers of genes in the human/mouse common signatures. There are several explanations to this finding. There is a contribution of the very high stringency of the "Min (test) vs. Max (ref)" ≥1x method that we used to establish the signatures, since any gene which was not consistently found overexpressed in all the replicates of all the species was excluded. As an example, the gene *DNAJC7*, identified as specific of pDC in our previous work (15) was removed from the human pDC signature because its "Min (test) vs. Max (ref)" ratio was equal to 0.933, due to a single lower human pDC replicate compared to a single replicate found with a higher signal in human MoDC. There is also a contribution of incomplete mapping of the genome of some of the species studied, leading to an underestimation of the number of orthologous genes that could be queried across all species. For example, *POU2F2*, more highly expressed in human and murine B cells as compared to many other immune cells, has not been mapped yet to the pig genome while it has been mapped to the genome of more distant species such as the spotted gar with a 1-to-1 orthology relationship. Another prominent cause is linked to technical limitations of the microarray approach, such as lack of ProbeSets against certain genes in certain species. This is notably the case for the gene *CLEC9A*, known to be specific of cDC1 but for which no ProbeSet exists in the human Affymetrix HG U133 plus2 gene chip. Sometimes, low signal-to-noise ratio for certain ProbeSets can also be responsible for the loss of putative interesting signature genes, such as *ZNF521* (*Zfp521* in mouse) found to be highly specific of pDC in mouse and human while the pig and sheep orthologous ProbeSet remains at the background level whatever the cell type considered. Recent technological advances now allow performing high throughput RNA sequencing at single cell levels with high sensitivity and processivity, which could solve most of the above issues; indeed, all expressed genes should be detected without any bias and analysis at the single cell level should alleviate any issue of cross-contamination between cell types. Therefore, the generation of gene-expression data for many individual cells of the same type should increase statistical power to define genes co-expressed at the single cell level and defining cell type-specific transcriptomic modules (22). Single cell gene-expression profiling recently allowed the unbiased and *de novo* identification of the different cell types of spleen (80) and central nervous system (81, 82) via the description of their molecular identity, starting from the bulk population of all the cells that could be extracted from the organ, without any prior enrichment procedure, based on the use of potentially confounding phenotypic marker combinations. However, this strategy is

TABLE 3 | Proposition of marker combination for oligo-phenotyping of mononuclear phagocytic cell subsets across species.

Exclusion	**Anti-CD3, anti-NK cells, and anti-B cells, if available[a]**						
Targeted cell population	pDC	cDC1	cDC2	MoDC	cMo	ncMo	MP
Combination of known markers[b]	$FLT3^+$ $SIRP\alpha^{lo}$ $MHC\text{-}II^{lo}$	$FLT3^{hi}$ $SIRP\alpha^{lo}$ $MHC\text{-}II^+$ $CD11c^+$ $CADM1^{hi}$	$FLT3^+$ $SIRP\alpha^+$ $MHC\text{-}II^+$ $CD11c^+$ $CADM1^{lo}$	$FLT3^-$ $SIRP\alpha^+$ $MHC\text{-}II^+$ $CD11c^+$	$FLT3^-$ $SIRP\alpha^+$	$FLT3^-$ $SIRP\alpha^+$	$FLT3^-$ $SIRP\alpha^+$
New additional candidates[c]	$LRP8^+$ $TSPAN13^{hi}$ $SLC30A5^+$ $NRP1^+$	$XCR1^+$	$SIGLEC8^+$ $IGSF6^+$	$FCGR2B^{hi}$ $CD200R1^+$	$CSF1R^{int}$ $CCR1^+$ $C19ORF59^+$	$CSF1R^{hi}$ $CD83^+$	$CSF1R^+$ $TLR4^{hi}$ $C5AR1^+$

[a] *Exclusion with anti-CD3, anti-NK cells, and anti-B cell markers is desirable when appropriate tools are available.*

[b] *A combination of known markers including FLT3, MHC-II, CD11c, SIRPα, and CADM1 allows a first step of identification of subset candidates but is at risk of contamination by sister cell types, or may be incomplete due to non-availability of one of the marker. FLT3 labeling may be performed by using recombinant His-tag FLT3L generated for the relevant species as recently proposed in a review (21).*

[c] *New additional candidate markers for refinement of subset identification are derived from the identification of genes encoding cell surface molecules from the conserved cell subset gene signatures.*

still extremely difficult to apply to species which genome has not yet been completely assembled, as well as to very rare cell types recovered upon prior phenotype-based enrichment. Moreover, to obtain information of sufficient completeness on functionally important genes for which few mRNA are expressed per cell, it is necessary to sequence at a sufficient depth of about one million reads per cell, which today still represents a very high cost when multiplied by the number of individual cells and conditions. Finally, the interpretation of the RNA-seq data on single cells is still largely based on the transcriptomic/molecular identity of cell types that are deduced from microarray analysis of purified cell pools. Hence, our work constitutes a major advancement in the field and is a necessary step before an eventual, later, refinement of the definition of cell subsets and their associated molecular signatures using single cell RNA-seq. The canonical gene-expression signatures that we generated can be used to distinguish and identify cell subsets in other vertebrate species. The cDC1 signature and the cDC2 vs. cDC1 signatures could be evaluated in chicken cDC sorted as single cells to determine whether this population includes only cDC1, as suggested by the trans-vertebrate hierarchical clustering, or a mixture of cDC1 and cDC2.

The conservation of gene signatures and interacting gene networks in homologous cell subsets throughout evolution is likely to bear strong biological meaning. Indeed, many genes of the conserved signatures were already known for their functions in these cells, validating the biological relevance of our signatures. In several instances, the same functional annotations were enriched in distinct subset signatures, but the genes responsible for the enrichments differed. For example, the genes responsible for the enrichment of the pathway "role of pattern recognition receptor in recognition of bacteria and viruses" were *TLR4*, *TLR8*, and *C5AR1* for the Mo/MP vs. cDC signature, *TLR2*, *CLEC7A*, *IL1B*, and *PIK3CB* for the cDC vs. pDC signature, and *TLR8*, *CLEC6A*, *DDX58*, *OAS2*, and *IL1B* for the cDC2 vs. (pDC and cDC1) signature. This analysis shows that cDC and MP express different sets of pattern recognition receptors for detection of viruses and bacteria, and that, within DC, cDC2 are also equipped differently from cDC1 and pDC for sensing of viruses and bacteria. These observations extended to other mammalian species the previous reports that human and mouse cDC2 are preferentially equipped with PRR targeting bacteria or involved in cytosolic sensing of viral infection (83, 84), and that TLR4 is very weakly expressed on pDC and cDC as compared to Mo/MP (83, 85). Similarly, different subset signatures were all enriched for "inflammatory response," "inflammation of organs," and "bacterial infection" but due to different genes. Altogether, this analysis indicates that different mononuclear phagocyte subsets express distinct and specific gene-expression modules which can sometimes contribute in a complementary way to the same general biological process in a conserved manner throughout evolution. Within the conserved gene-expression programs in mononuclear phagocyte subsets, we identified novel candidate genes and putative upstream regulators which likely contribute to the control of the ontogeny or functions of the corresponding cell type. For instance, the *FNBP1* and *SNX22* encoded proteins may be involved in the specific intracellular trafficking properties promoting antigen cross-presentation by cDC1, *ARHGAP22* and *NAV1* could modulate the organization of the cytoskeleton of cDC to control their mobility or antigen presentation functions, and the transcription regulators *BCL11A* and *MSL2A* may control specific gene networks in cDC. *BLC11A* is known to be key in murine pDC development (50) but it may have a specific role in cDC homeostasis, as inferred from a previous study (86). Our study thus opens the way for deciphering the sets of genes encoding functional cellular modules and their specifying transcription factors in subsets of mononuclear cells, in order to further improve and connect together the molecular and functional definitions of these cell types across species (22, 23).

Conclusion

Our meta-analysis that combines cell sorting and comparative transcriptomic analysis was implemented as a methodology pipeline that could be used by biologists with minimal training in bio-informatics for subsequent extension to other species and to other complex cellular systems. Our study should lead to the identification of homologous mononuclear phagocyte subsets in species other than sheep and pigs, and which are of importance for biomedical investigations, such as bats, rabbits, ferrets, guinea pigs, possibly zebrafishes, and in species of veterinary importance including pets and animals of the food economy. The characterization of mononuclear phagocyte subsets in these species will allow manipulating their immune responses against diseases for the sustainability of our environment.

Author Contributions

ISC and MD directed research and wrote the paper with input from TPVM. TPVM carried out bio-informatics analyses with input from MD. JEY performed most of the cell purification experiments and analyzed data, with input from CU (microarray hybridization, blood processing), SR (pDC isolation), MB (cell sorting), MM (microarray hybridization and analysis), HM (chicken array data), PQ (chicken array data), NB (pig cell phenotyping). PB performed phylogenetic analyses. GF and HS provided key cell types (MoDC) and reagents (unique mAb, non-commercially available). LJ performed array annotations.

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Supplementary Material

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2015.00299

References

1. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* (2010) **116**(16):e74–80. doi:10.1182/blood-2010-02-258558
2. Mildner A, Yona S, Jung S. A close encounter of the third kind: monocyte-derived cells. *Adv Immunol* (2013) **120**:69–103. doi:10.1016/B978-0-12-417028-5.00003-X
3. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* (2013) **39**(5):925–38. doi:10.1016/j.immuni.2013.10.004
4. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* (2014) **15**(10):929–37. doi:10.1038/ni.2967
5. McGovern N, Schlitzer A, Gunawan M, Jardine L, Shin A, Poyner E, et al. Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages. *Immunity* (2014) **41**(3):465–77. doi:10.1016/j.immuni.2014.08.006
6. Molawi K, Wolf Y, Kandalla PK, Favret J, Hagemeyer N, Frenzel K, et al. Progressive replacement of embryo-derived cardiac macrophages with age. *J Exp Med* (2014) **211**(11):2151–8. doi:10.1084/jem.20140639
7. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* (2014) **159**(6):1312–26. doi:10.1016/j.cell.2014.11.018
8. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* (2014) **159**(6):1327–40. doi:10.1016/j.cell.2014.11.023
9. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**(11):1118–28. doi:10.1038/ni.2419
10. Cheong C, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP, et al. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* (2010) **143**(3):416–29. doi:10.1016/j.cell.2010.09.039
11. Xu Y, Zhan Y, Lew AM, Naik SH, Kershaw MH. Differential development of murine dendritic cells by GM-CSF versus Flt3 ligand has implications for inflammation and trafficking. *J Immunol* (2007) **179**(11):7577–84. doi:10.4049/jimmunol.179.11.7577
12. Mayer CT, Ghorbani P, Nandan A, Dudek M, Arnold-Schrauf C, Hesse C, et al. Selective and efficient generation of functional Batf3-dependent CD103+ dendritic cells from mouse bone marrow. *Blood* (2014) **24**:3081–91. doi:10.1182/blood-2013-12-545772
13. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-derived interleukin-34 controls the development and maintenance of Langerhans cells and the maintenance of microglia. *Immunity* (2012) **37**(6):1050–60. doi:10.1016/j.immuni.2012.11.001
14. Schlitzer A, Ginhoux F. Organization of the mouse and human DC network. *Curr Opin Immunol* (2014) **26**:90–9. doi:10.1016/j.coi.2013.11.002
15. Robbins SH, Walzer T, Dembele D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* (2008) **9**(1):R17. doi:10.1186/gb-2008-9-1-r17
16. Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, et al. Human tissues contain CD141(hi) cross-presenting dendritic cells with functional homology to mouse CD103(+) nonlymphoid dendritic cells. *Immunity* (2012) **37**(1):60–73. doi:10.1016/j.immuni.2012.04.012
17. Watchmaker PB, Lahl K, Lee M, Baumjohann D, Morton J, Kim SJ, et al. Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. *Nat Immunol* (2014) **15**(1):98–108. doi:10.1038/ni.2768
18. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* (2010) **33**(3):375–86. doi:10.1016/j.immuni.2010.08.012
19. Crozat K, Guiton R, Guilliams M, Henri S, Baranek T, Schwartz-Cornil I, et al. Comparative genomics as a tool to reveal functional equivalences between human and mouse dendritic cell subsets. *Immunol Rev* (2010) **234**(1):177–98. doi:10.1111/j.0105-2896.2009.00868.x
20. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. *Blood* (2010) **115**(3):e10–9. doi:10.1182/blood-2009-07-235028
21. Summerfield A, Auray G, Ricklin M. Comparative dendritic cell biology of veterinary mammals. *Annu Rev Anim Biosci* (2014) **3**:533–57. doi:10.1146/annurev-animal-022114-111009
22. Achim K, Arendt D. Structural evolution of cell types by step-wise assembly of cellular modules. *Curr Opin Genet Dev* (2014) **27**:102–8. doi:10.1016/j.gde.2014.05.001
23. Arendt D. The evolution of cell types in animals: emerging principles from molecular studies. *Nat Rev Genet* (2008) **9**(11):868–82. doi:10.1038/nrg2416
24. Chevallier N, Berthelemy M, Le Rhun D, Laine V, Levy D, Schwartz-Cornil I. Bovine leukemia virus-induced lymphocytosis and increased cell survival mainly involve the CD11b+ B-lymphocyte subset in sheep. *J Virol* (1998) **72**(5):4413–20.
25. Fiebach AR, Guzylack-Piriou L, Python S, Summerfield A, Ruggli N. Classical swine fever virus N(pro) limits type I interferon induction in plasmacytoid dendritic cells by interacting with interferon regulatory factor 7. *J Virol* (2011) **85**(16):8002–11. doi:10.1128/JVI.00330-11
26. Ruscanu S, Pascale F, Bourge M, Hemati B, Elhmouzi-Younes J, Urien C, et al. The double-stranded RNA bluetongue virus induces type I interferon in plasmacytoid dendritic cells via a MYD88-dependent TLR7/8-independent signaling pathway. *J Virol* (2012) **86**(10):5817–28. doi:10.1128/JVI.06716-11
27. Foulon E, Foucras G. Two populations of ovine bone marrow-derived dendritic cells can be generated with recombinant GM-CSF and separated on CD11b expression. *J Immunol Methods* (2008) **339**(1):1–10. doi:10.1016/j.jim.2008.07.012
28. Ruscanu S, Jouneau L, Urien C, Bourge M, Lecardonnel J, Moroldo M, et al. Dendritic cell subtypes from lymph nodes and blood show contrasted gene expression programs upon bluetongue virus infection. *J Virol* (2013) **87**(16):9333–43. doi:10.1128/JVI.00631-13
29. Marquet F, Vu Manh TP, Maisonnasse P, Elhmouzi-Younes J, Urien C, Bouguyon E, et al. Pig skin includes dendritic cell subsets transcriptomically related to human CD1a and CD14 dendritic cells presenting different migrating behaviors and T cell activation capacities. *J Immunol* (2014) **193**(12):5883–93. doi:10.4049/jimmunol.1303150
30. Casel P, Moreews F, Lagarrigue S, Klopp C. sigReannot: an oligo-set re-annotation pipeline based on similarities with the ensemble transcripts and unigene clusters. *BMC Proc* (2009) **3**(Suppl 4):S3. doi:10.1186/1753-6561-3-S4-S3
31. Vu Manh TP, Marty H, Sibille P, Le Vern Y, Kaspers B, Dalod M, et al. Existence of conventional dendritic cells in *Gallus gallus* revealed by comparative gene expression profiling. *J Immunol* (2014) **192**(10):4510–7. doi:10.4049/jimmunol.1303405
32. Baranek T, Vu Manh TP, Alexandre Y, Maqbool MA, Cabeza JZ, Tomasello E, et al. Differential responses of immune cells to type I interferon contribute to host resistance to viral infection. *Cell Host Microbe* (2012) **12**(4):571–84. doi:10.1016/j.chom.2012.09.002
33. Subramanian A, Kuehn H, Gould J, Tamayo P, Mesirov JP. GSEA-P: a desktop application for gene set enrichment analysis. *Bioinformatics* (2007) **23**(23):3251–3. doi:10.1093/bioinformatics/btm369
34. Gurka S, Hartung E, Becker M, Kroczek RA. Mouse conventional dendritic cells can be universally classified based on the mutually exclusive expression of XCR1 and SIRPa. *Front Immunol* (2015) **6**:35. doi:10.3389/fimmu.2015.00035

35. Dorner BG, Dorner MB, Zhou X, Opitz C, Mora A, Guttler S, et al. Selective expression of the chemokine receptor XCR1 on cross-presenting dendritic cells determines cooperation with CD8+ T cells. *Immunity* (2009) **31**(5):823–33. doi:10.1016/j.immuni.2009.08.027
36. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, Ventre E, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8{alpha}+ dendritic cells. *J Exp Med* (2010) **207**:1283–92. doi:10.1084/jem.20100223
37. Bachem A, Guttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J Exp Med* (2010) **207**(6):1273–81. doi:10.1084/jem.20100348
38. Crozat K, Tamoutounour S, Vu Manh TP, Fossum E, Luche H, Ardouin L, et al. Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the CD8{alpha}+ type. *J Immunol* (2011) **187**(9):4411–5. doi:10.4049/jimmunol.1101717
39. Bachem A, Hartung E, Guttler S, Mora A, Zhou X, Hegemann A, et al. Expression of XCR1 characterizes the Batf3-dependent lineage of dendritic cells capable of antigen cross-presentation. *Front Immunol* (2012) **3**:214. doi:10.3389/fimmu.2012.00214
40. Balan S, Ollion V, Colletti N, Chelbi R, Montanana-Sanchis F, Liu H, et al. Human XCR1+ dendritic cells derived in vitro from CD34+ progenitors closely resemble blood dendritic cells, including their adjuvant responsiveness, contrary to monocyte-derived dendritic cells. *J Immunol* (2014) **193**(4):1622–35. doi:10.4049/jimmunol.1401243
41. Dutertre CA, Jourdain JP, Rancez M, Amraoui S, Fossum E, Bogen B, et al. TLR3-responsive, XCR1+, CD141(BDCA-3)+/CD8alpha+-equivalent dendritic cells uncovered in healthy and simian immunodeficiency virus-infected rhesus macaques. *J Immunol* (2014) **192**(10):4697–708. doi:10.4049/jimmunol.1302448
42. Contreras V, Urien C, Guiton R, Alexandre Y, Vu Manh TP, Andrieu T, et al. Existence of CD8{alpha}-like dendritic cells with a conserved functional specialization and a common molecular signature in distant mammalian species. *J Immunol* (2010) **185**:3313–25. doi:10.4049/jimmunol.1000824
43. Dutertre CA, Wang LF, Ginhoux F. Aligning bona fide dendritic cell populations across species. *Cell Immunol* (2014) **291**(1–2):3–10. doi:10.1016/j.cellimm.2014.08.006
44. Guilliams M, Henri S, Tamoutounour S, Ardouin L, Schwartz-Cornil I, Dalod M, et al. From skin dendritic cells to a simplified classification of human and mouse dendritic cell subsets. *Eur J Immunol* (2010) **40**(8):2089–94. doi:10.1002/eji.201040498
45. Hajjoubi S, Rival-Gervier S, Hayes H, Floriot S, Eggen A, Piumi F, et al. Ruminants genome no longer contains whey acidic protein gene but only a pseudogene. *Gene* (2006) **370**:104–12. doi:10.1016/j.gene.2005.11.025
46. de Jong WW, van Dijk MA, Poux C, Kappe G, van Rheede T, Madsen O. Indels in protein-coding sequences of euarchontoglires constrain the rooting of the eutherian tree. *Mol Phylogenet Evol* (2003) **28**(2):328–40. doi:10.1016/S1055-7903(03)00116-7
47. Douady CJ, Douzery EJ. Molecular estimation of eulipotyphlan divergence times and the evolution of "insectivore". *Mol Phylogenet Evol* (2003) **28**(2):285–96. doi:10.1016/S1055-7903(03)00119-2
48. Pascale F, Contreras V, Bonneau M, Courbet A, Chilmonczyk S, Bevilacqua C, et al. Plasmacytoid dendritic cells migrate in afferent skin lymph. *J Immunol* (2008) **180**(9):5963–72. doi:10.4049/jimmunol.180.9.5963
49. Summerfield A, Guzylack-Piriou L, Schaub A, Carrasco CP, Tache V, Charley B, et al. Porcine peripheral blood dendritic cells and natural interferon-producing cells. *Immunology* (2003) **110**(4):440–9. doi:10.1111/j.1365-2567.2003.01755.x
50. Ippolito GC, Dekker JD, Wang YH, Lee BK, Shaffer AL III, Lin J, et al. Dendritic cell fate is determined by BCL11A. *Proc Natl Acad Sci U S A* (2014) **111**(11):E998–1006. doi:10.1073/pnas.1319228111
51. Cao T, Ueno H, Glaser C, Fay JW, Palucka AK, Banchereau J. Both Langerhans cells and interstitial DC cross-present melanoma antigens and efficiently activate antigen-specific CTL. *Eur J Immunol* (2007) **37**(9):2657–67. doi:10.1002/eji.200636499
52. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* (2008) **135**(1):37–48. doi:10.1016/j.cell.2008.09.016
53. Fredebohm J, Wolf J, Hoheisel JD, Boettcher M. Depletion of RAD17 sensitizes pancreatic cancer cells to gemcitabine. *J Cell Sci* (2013) **126**(Pt 15):3380–9. doi:10.1242/jcs.124768
54. Bloch DB, de la Monte SM, Guigaouri P, Filippov A, Bloch KD. Identification and characterization of a leukocyte-specific component of the nuclear body. *J Biol Chem* (1996) **271**(46):29198–204. doi:10.1074/jbc.271.46.29198
55. Hirano M, Guo P, McCurley N, Schorpp M, Das S, Boehm T, et al. Evolutionary implications of a third lymphocyte lineage in lampreys. *Nature* (2013) **501**(7467):435–8. doi:10.1038/nature12467
56. Sawai CM, Sisirak V, Ghosh HS, Hou EZ, Ceribelli M, Staudt LM, et al. Transcription factor Runx2 controls the development and migration of plasmacytoid dendritic cells. *J Exp Med* (2013) **210**(11):2151–9. doi:10.1084/jem.20130443
57. Iwakoshi NN, Pypaert M, Glimcher LH. The transcription factor XBP-1 is essential for the development and survival of dendritic cells. *J Exp Med* (2007) **204**(10):2267–75. doi:10.1084/jem.20070525
58. Cao W, Zhang L, Rosen DB, Bover L, Watanabe G, Bao M, et al. BDCA2/Fc epsilon RI gamma complex signals through a novel BCR-like pathway in human plasmacytoid dendritic cells. *PLoS Biol* (2007) **5**(10):e248. doi:10.1371/journal.pbio.0050248
59. Haniffa M, Collin M, Ginhoux F. Ontogeny and functional specialization of dendritic cells in human and mouse. *Adv Immunol* (2013) **120**:1–49. doi:10.1016/B978-0-12-417028-5.00001-6
60. Murphy TL, Tussiwand R, Murphy KM. Specificity through cooperation: BATF-IRF interactions control immune-regulatory networks. *Nat Rev Immunol* (2013) **13**(7):499–509. doi:10.1038/nri3470
61. Mori M, Saito K, Ohta Y. ARHGAP22 localizes at endosomes and regulates actin cytoskeleton. *PLoS One* (2014) **9**(6):e100271. doi:10.1371/journal.pone.0100271
62. Yang W, Shen N, Ye DQ, Liu Q, Zhang Y, Qian XX, et al. Genome-wide association study in Asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus. *PLoS Genet* (2010) **6**(2):e1000841. doi:10.1371/journal.pgen.1000841
63. Tsujita K, Kondo A, Kurisu S, Hasegawa J, Itoh T, Takenawa T. Antagonistic regulation of F-BAR protein assemblies controls actin polymerization during podosome formation. *J Cell Sci* (2013) **126**(Pt 10):2267–78. doi:10.1242/jcs.122515
64. Worby CA, Dixon JE. Sorting out the cellular functions of sorting nexins. *Nat Rev Mol Cell Biol* (2002) **3**(12):919–31. doi:10.1038/nrm974
65. Shih VF, Davis-Turak J, Macal M, Huang JQ, Ponomarenko J, Kearns JD, et al. Control of RelB during dendritic cell activation integrates canonical and noncanonical NF-kappaB pathways. *Nat Immunol* (2012) **13**(12):1162–70. doi:10.1038/ni.2446
66. Singh R, Cresswell P. Defective cross-presentation of viral antigens in GILT-free mice. *Science* (2010) **328**(5984):1394–8. doi:10.1126/science.1189176
67. West LC, Grotzke JE, Cresswell P. MHC class II-restricted presentation of the major house dust mite allergen Der p 1 Is GILT-dependent: implications for allergic asthma. *PLoS One* (2013) **8**(1):e51343. doi:10.1371/journal.pone.0051343
68. Mihrshahi R, Barclay AN, Brown MH. Essential roles for Dok2 and RasGAP in CD200 receptor-mediated regulation of human myeloid cells. *J Immunol* (2009) **183**(8):4879–86. doi:10.4049/jimmunol.0901531
69. Moniuszko M, Liyanage NP, Doster MN, Parks RW, Grubczak K, Lipinska D, et al. Glucocorticoid treatment at moderate doses of SIV-infected rhesus macaques decreases the frequency of circulating CD14CD16 monocytes but does not alter the tissue virus reservoir. *AIDS Res Hum Retroviruses* (2015) **31**(1):115–26. doi:10.1089/AID.2013.0220
70. Fingerle-Rowson G, Angstwurm M, Andreesen R, Ziegler-Heitbrock HW. Selective depletion of CD14+ CD16+ monocytes by glucocorticoid therapy. *Clin Exp Immunol* (1998) **112**(3):501–6. doi:10.1046/j.1365-2249.1998.00617.x
71. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* (2008) **322**(5904):1097–100. doi:10.1126/science.1164206
72. Kasahara M, Sutoh Y. Two forms of adaptive immunity in vertebrates: similarities and differences. *Adv Immunol* (2014) **122**:59–90. doi:10.1016/B978-0-12-800267-4.00002-X
73. Irion U, Krauss J, Nusslein-Volhard C. Precise and efficient genome editing in zebrafish using the CRISPR/Cas9 system. *Development* (2014) **141**(24):4827–30. doi:10.1242/dev.115584
74. Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, Matusik R, et al. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. *Cancer Cell* (2003) **4**(3):223–38. doi:10.1016/S1535-6108(03)00197-1
75. Lee JS, Chu IS, Mikaelyan A, Calvisi DF, Heo J, Reddy JK, et al. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. *Nat Genet* (2004) **36**(12):1306–11. doi:10.1038/ng1481

76. Vu Manh TP, Alexandre Y, Baranek T, Crozat K, Dalod M. Plasmacytoid, conventional, and monocyte-derived dendritic cells undergo a profound and convergent genetic reprogramming during their maturation. *Eur J Immunol* (2013) **43**(7):1706–15. doi:10.1002/eji.201243106
77. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol* (2014) **32**:227–55. doi:10.1146/annurev-immunol-032713-120225
78. Artyomov MN, Munk A, Gorvel L, Korenfeld D, Cella M, Tung T, et al. Modular expression analysis reveals functional conservation between human Langerhans cells and mouse cross-priming dendritic cells. *J Exp Med* (2015) **212**(5):743–57. doi:10.1084/jem.20131675
79. Fairbairn L, Kapetanovic R, Beraldi D, Sester DP, Tuggle CK, Archibald AL, et al. Comparative analysis of monocyte subsets in the pig. *J Immunol* (2013) **190**(12):6389–96. doi:10.4049/jimmunol.1300365
80. Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science* (2014) **343**(6172):776–9. doi:10.1126/science.1247651
81. Usoskin D, Furlan A, Islam S, Abdo H, Lonnerberg P, Lou D, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* (2015) **18**(1):145–53. doi:10.1038/nn.3881
82. Zeisel A, Manchado ABM, Codeluppi S, Lönnerberg P, La Manno G, Juréus A, et al. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science* (2015) **347**(6226):1138–42. doi:10.1126/science.aaa1934
83. Crozat K, Vivier E, Dalod M. Crosstalk between components of the innate immune system: promoting anti-microbial defenses and avoiding immunopathologies. *Immunol Rev* (2009) **227**(1):129–49. doi:10.1111/j.1600-065X.2008.00736.x
84. Luber CA, Cox J, Lauterbach H, Fancke B, Selbach M, Tschopp J, et al. Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity* (2010) **32**(2):279–89. doi:10.1016/j.immuni.2010.01.013
85. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* (2012) **13**(9):888–99. doi:10.1038/ni.2370
86. Wu X, Satpathy AT, Kc W, Liu P, Murphy TL, Murphy KM. Bcl11a controls Flt3 expression in early hematopoietic progenitors and is required for pDC development in vivo. *PLoS One* (2013) **8**(5):e64800. doi:10.1371/journal.pone.0064800

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Investigating evolutionary conservation of dendritic cell subset identity and functions

*Thien-Phong Vu Manh[1,2,3], Nicolas Bertho[4], Anne Hosmalin[5,6,7,8], Isabelle Schwartz-Cornil[4] and Marc Dalod[1,2,3]**

[1] UM2, Centre d'Immunologie de Marseille-Luminy (CIML), Aix-Marseille University, Marseille, France, [2] U1104, Institut National de la Santé et de la Recherche Médicale (INSERM), Marseille, France, [3] UMR7280, Centre National de la Recherche Scientifique (CNRS), Marseille, France, [4] Virologie et Immunologie Moléculaires UR892, Institut National de la Recherche Agronomique, Jouy-en-Josas, France, [5] INSERM U1016, Institut Cochin, Paris, France, [6] CNRS UMR8104, Paris, France, [7] Université Paris Descartes, Paris, France, [8] Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Cochin, Paris, France

Dendritic cells (DCs) were initially defined as mononuclear phagocytes with a dendritic morphology and an exquisite efficiency for naïve T-cell activation. DC encompass several subsets initially identified by their expression of specific cell surface molecules and later shown to excel in distinct functions and to develop under the instruction of different transcription factors or cytokines. Very few cell surface molecules are expressed in a specific manner on any immune cell type. Hence, to identify cell types, the sole use of a small number of cell surface markers in classical flow cytometry can be deceiving. Moreover, the markers currently used to define mononuclear phagocyte subsets vary depending on the tissue and animal species studied and even between laboratories. This has led to confusion in the definition of DC subset identity and in their attribution of specific functions. There is a strong need to identify a rigorous and consensus way to define mononuclear phagocyte subsets, with precise guidelines potentially applicable throughout tissues and species. We will discuss the advantages, drawbacks, and complementarities of different methodologies: cell surface phenotyping, ontogeny, functional characterization, and molecular profiling. We will advocate that gene expression profiling is a very rigorous, largely unbiased and accessible method to define the identity of mononuclear phagocyte subsets, which strengthens and refines surface phenotyping. It is uniquely powerful to yield new, experimentally testable, hypotheses on the ontogeny or functions of mononuclear phagocyte subsets, their molecular regulation, and their evolutionary conservation. We propose defining cell populations based on a combination of cell surface phenotyping, expression analysis of hallmark genes, and robust functional assays, in order to reach a consensus and integrate faster the huge but scattered knowledge accumulated by different laboratories on different cell types, organs, and species.

Keywords: mononuclear phagocytes, comparative genomics, human, non-human primates, mouse, pig, sheep, chicken

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****Correspondence:***
Marc Dalod, Centre d'Immunologie de Marseille-Luminy (CIML), Parc Scientifique et Technologique de Luminy, Case 906, Marseille Cedex 09 F-13288, France
dalod@ciml.univ-mrs.fr

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Introduction

The immune system includes a large variety of myeloid and lymphoid cell types which develop through distinct ontogenic pathways, express specific phenotypes, and exert specialized functions.

The mononuclear phagocytes form a complex group of myeloid cells that encompass three major cell types, i.e., monocytes, macrophages, and dendritic cells (DC), together with their proximal progenitors. These three cell types contribute to maintain host integrity by shaping the innate and adaptive immune defense, a generic function related to their common phagocytic properties and their capacity to present antigen to T cells. These functions are also shared by other types of professional antigen-presenting cells (APCs), in particular B lymphocytes. However, different types of APCs are primarily devoted to distinct functions (**Figure 1**). B cells produce antibodies. Monocytes patrol the organism for the detection of pathogens and dominantly display inflammatory and oxidative stress response. Macrophages mainly perform microbicidal, scavenging, and tissue trophic/maintenance functions. DC are uniquely efficient for antigen-specific activation of naïve T lymphocytes, a process called T-cell priming. Indeed, DC were initially defined by their dendritic morphology and their exquisite capacity for T-cell priming. DC include two main cell types, the plasmacytoid DC (pDC) that are expert in type I interferon synthesis upon viral stimulation and the conventional DC (cDC) that are specialized in antigen capture, processing, and presentation for T-cell priming. Two cDC subsets can be distinguished based on a further segregation of functions. $XCR1^+$ cDC1 are particularly efficient in $CD8^+$ T-cell activation and cross-presentation, at least in mice. $XCR1^-$ cDC2 are most efficient for T helper cell priming, in particular polarization toward Th2 or Th17, and for the promotion of humoral immunity. Importantly, an additional layer of complexity is generated by the plasticity of the different mononuclear cell types, which display modified phenotypes and functions contingent to the anatomical microenvironment where they reside or when exposed to pathogens or inflammation. For instance, monocytes adopt a dendritic morphology and antigen-presentation functions in inflammatory settings (1–3) as well as when located in the dermis (4–6), leading to their designation as monocyte-derived DC (MoDC). Langerhans cells, long considered to be DC due to their morphology and antigen-presentation function, are now known as a type of tissue macrophages (7–13). More generally, the gene expression programs, phenotypes, and functional properties of macrophages are strongly influenced by their tissue of residence. Finally, not only $XCR1^+$ cDC but also other DC subsets including pDC and $XCR1^-$ cDC can also efficiently cross-present antigens to $CD8^+$ T cells when appropriately stimulated (14–22). Thus, the plasticity of the mononuclear phagocyte responses superimposes onto the segregation of phenotypes and functions attributed to subsets (**Figure 2**), which can lead to confusion in the definition of the different cell types if only based on functional assays. Hence, morphologic, phenotypic, and functional criteria are not sufficient to rigorously define mononuclear phagocyte subsets, and to properly discriminate what are distinct cell types as opposed to different developmental or activation states of a given cell type. Complementary or robust alternative criteria are needed to rigorously define the identity of the mononuclear phagocyte subsets.

Mononuclear phagocyte subsets were recently shown to develop from distinct progenitors and/or under the instruction of different transcription factors or cytokines. cDC and pDC derive from a dedicated bone marrow precursor, the common DC progenitor, with a differentiation potential strictly restricted to this hematopoietic lineage. pDC and cDC homeostasis exquisitely depends on the growth factor FLT3-L. pDC development strictly depends on the transcription factors TCF4 (E2-2) and SPIB both in mouse and human, $XCR1^+$ cDC development on the master transcription factor IRF8 at least in mice, and $XCR1^-$ cDC development on IRF4. Macrophages derive from a monocytic precursor, either of embryonic origin as in the case of Langerhans cells and microglia, or at least in part from circulating blood monocytes as in the case of gut macrophages. Egress of classical monocytes from the bone marrow into the blood strictly depends on the chemokine receptor CCR2. As a consequence, in competitive mixed bone marrow reconstitution experiments in mice, all cell types derived from circulating blood monocytes are primarily reconstituted from wild-type cells and not from CCR2-deficient cells. Hence, it has been proposed that the study of their developmental pathway, in other words ontogeny, was the best way to classify mononuclear phagocyte cell types, at least in the mouse model where the knowledge in DC subset properties is also the most advanced. Indeed, in this model, genetically modified animals unambiguously permit to track the development of cell types and to dissect their phenotypes and functions, in different contexts *in vivo*. However, the identity and functions of the different mononuclear phagocyte subsets need to be established outside of the mouse model, in animal species where ontogenic studies cannot be easily conducted, in order to accelerate translation of our advanced knowledge on the functioning of the mouse immune

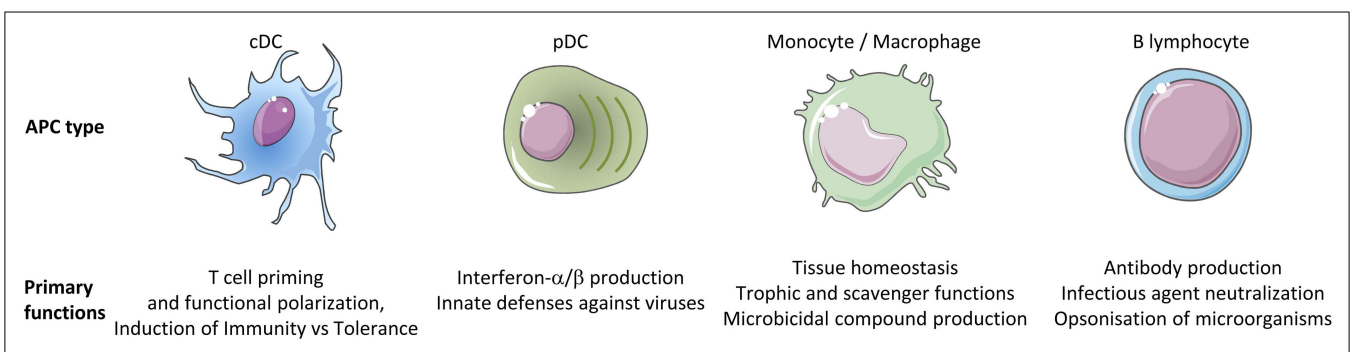

FIGURE 1 | Different types of APCs are specialized in distinct primary functions. cDC are uniquely efficient for the priming and functional polarization of T cells. Although other APCs also contribute to this process, this does not represent their primary functions. Hence, cDC play a central and non-redundant role in the orchestration of adaptive immunity.

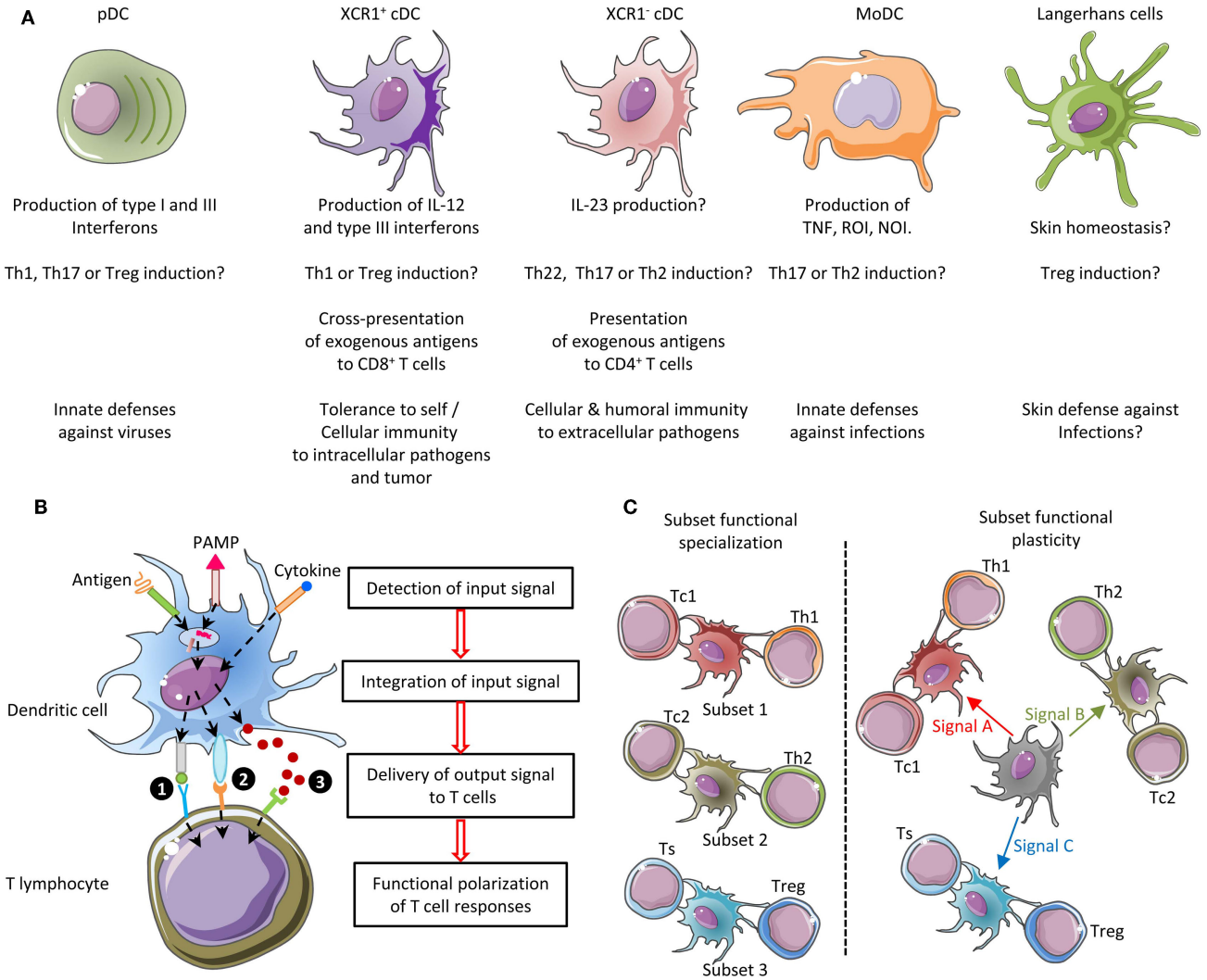

FIGURE 2 | Combined functional specialization and plasticity of DC subsets allows mounting different types of adaptive immune responses adapted to the various natures of the threats to be faced. **(A)** Five DC subsets can be defined in mice based in part on their functional specialization: pDC, XCR1$^+$ cDC, XCR1$^-$ cDC, MoDC, and Langerhans cells. Certain DC subsets are more efficient than others to exert a specific function, because they are intrinsically genetically built to activate this function faster and in more diverse settings. **(B)** The function of each DC subset is relatively plastic. Three types of output signals are delivered by DC to T cells and instruct their functional polarization: (1) ligands for the T-cell receptor (antigenic peptides presented in association with MHC molecules), (2) co-stimulation, and (3) cytokines. Co-stimulation and cytokine signals can be either activating (e.g., CD86 and IL-12, respectively) or inhibitory (e.g., PD-L1 and IL-10, respectively). Different cytokines induce distinct types of helper T-cell responses. For example, IL-12 primarily promotes Th1, IL-4 promotes Th2, and IL-23 promotes Th17. Each DC subset can sense a specific array of microbial or danger signals. Integration of the particular combination of input signals received by the DC in a given pathophysiological context determines the precise type of maturation ensuing and hence the combination of output signals delivered to T cells. As a result, different DC subsets can exert similar or complementary functions depending on the physiopathological context. **(C)** The combination of functional specialization and plasticity of subsets allows DC responses to be highly flexible and thus to react rapidly to different threats by coupling the type of danger sensed to the most appropriate type of immune response to induce for protection. However, this flexibility can lead to confusion if attempting to define DC subsets only on functional specialization. NOI, nitric oxide intermediates; ROI, radical oxygen intermediates; Th, T helper cell; Tc, cytotoxic T cell; Treg, regulatory T cell; Ts, T suppressor cell.

system toward clinical and/or economical applications to sustain global human health. Very promising vaccine and immunomodulatory strategies have been developed in mouse models based on DC subset targeting (23–35). The translation of these strategies to human and other species has not yet reached the expected success, likely due to insufficient knowledge in the identity and function of homologous DC subsets across species. This knowledge is needed in biomedical model species, primarily in non-human primates, and also in alternative models such as pigs that share physiological and anatomical similarities with humans – for instance skin and lung structural properties – and that present sensitivity to human pathogens of great importance for public health such as influenza. In addition, this knowledge is needed for companion and sport animals, and for animals of the agro-economy, such as ruminants, pigs, poultry, and fishes, with the goal to improve vaccination strategies against pathogens responsible for major

economic losses, to decrease antibiotic use and to ameliorate animal welfare. These species, as well as wild animals, are also targets or reservoirs for major zoonotic pathogens whose control could thus benefit from new vaccine strategies targeting DC subsets in these animal species. This raises the question how to best define DC subset identity and functions in a way that can be extrapolated from mouse to human and other species, for clinical applications as well as for a better understanding of the evolution of the immune system.

Different Methodologies to Define the Identity of Immune Cell Types, with Their Advantages and Drawbacks

Several methodologies have been proposed to define cell types. They include cell surface phenotyping and morphology, ontogeny, functional characterization, molecular profiling at population level, and molecular profiling at single cell level. We will discuss the specific drawbacks and advantages of each of these approaches (**Table 1**).

Cell Surface Phenotyping and Morphology

Cell surface phenotyping generally is a mandatory first step for all other proposed methodologies aiming at defining DC subsets. It may be skipped only for particular experiments of molecular profiling at single cell level and perhaps for functional tests based on validated protocols for specific depletion of the targeted cell subset *in vivo*. Indeed, phenotypic characterization/identification of DC subsets is necessary either to purify them for morphological analysis, functional assays, or molecular profiling, or to compare their characteristics in tissues or bulk cell suspensions (expression of lineage reporters in cell fate mapping experiments, anatomical location, maturation status, cytokine production, interactions with T cells. . .). Phenotypic characterization through cell surface phenotyping by flow cytometry is the method of DC subset identification the easiest to perform and the most frequently used. No single cell surface marker has been found to be sufficient for identification of a given DC subset, except for XCR1 expression on mouse and human XCR1$^+$ cDC (18, 36–42) and maybe BDCA2 or LILRA4 expression on human pDC (43–46). Thus, to rigorously identify any given DC subset in any species with a limited risk of contamination by another cell type, most of the time complex combinations of multiple markers are required, often including the use of exclusion marker to ensure lack of contamination of the cell population targeted by other cell types sharing with it many positive markers. For example, the CD8α^+ subset of mouse pDC can heavily contaminate mouse lymphoid organ-resident XCR1$^+$ cDC when defined phenotypically as Lineage$^-$ CD11c$^+$CD8α^+ (47–49). This problem can be solved by exclusion of SiglecH$^+$ or CCR9$^+$ cells or by using XCR1 as a positive marker. Similarly, other cells including MoDC or activated CD1c (BDCA1)$^+$ XCR1$^-$ cDC can heavily contaminate human XCR1$^+$ cDC when defined phenotypically as Lineage$^-$ HLA-DR$^+$CD141 (BDCA3)$^+$ (41, 50, 51). This problem can be solved by using CADM1 or XCR1 as additional positive markers (41, 52). Rigorous phenotypic identification of XCR1$^-$ cDC (mouse CD11b$^+$ cDC and human CD1c$^+$ cDC) can be much more challenging, since these cells can be difficult to discriminate from MoDC, in particular under inflammation settings (53, 54). Identification of DC based on oligoparameter phenotyping is even more at risk of inaccuracy in other species, due to the limited panel of available antibodies directed to surface markers and to the poor knowledge in surface marker expression selectivity in non-DC cell types. However, major advances have recently been made to refine strategies for DC subset identification by cell surface phenotyping, in part based on novel knowledge gained through ontogeny and molecular profiling studies as will be discussed below. Hence, protocols for DC subset identification by cell surface phenotyping might soon become standardized, at least in mouse and human. This would allow better comparison of data across laboratories and limit the risk of use of inappropriate protocols leading to improper data interpretation. Special attention should be given to enzymatic dissociation that can strongly modify cell surface marker detection. Ideally, universal phenotyping protocols could be designed, allowing to considerably simplify the current nomenclatures for DC subsets by using the same name and similar marker combinations to identify homologous cell types irrespective of their tissues and species of origin (55–57). Moreover, the markers used to define and name DC subsets could be chosen based on their relevance to the biology of these cells, contrary to the current situation where the markers used were discovered fortuitously/empirically and may not be linked to the biology of the eponymous cells, as is the case for CD8α and CD141 for mouse and human XCR1$^+$ cDC, respectively. However, when identifying a potentially new subset of DC or studying in a novel context a potentially known DC subset, a number of precautions need to be taken for data interpretation, including confirmation of conclusions by complementary methods such as ontogeny, functional, or molecular profiling studies.

Ontogeny

Ontogeny studies in mice, in particular studies on the dependence of DC subset development on transcription factors, have been instrumental in identifying the homologies between lymphoid tissue-resident CD8α^+ cDC and the CD103$^+$CD11b$^-$ cDC present in non-lymphoid tissues and migrating into the draining lymph nodes once activated (58). These studies, together with gene expression profiling analyses (9, 40), ultimately allowed grouping mouse CD8α^+ cDC and CD103$^+$CD11b$^-$ cDC together under the umbrella of the XCR1$^+$ cDC subset (38, 40, 59, 60). The recent discrimination of mouse CD11b$^+$ cDC from MoDC has also been largely based on the analysis of the role of specific chemokine or growth factor receptors on cell type development *in vivo*, namely CCR2 dependence as a characteristic of monocytic origin and FLT3 dependence as a proof of cDC identity (2, 3, 6, 61). In addition, mouse CD11b$^+$ cDC development was shown to selectively depend on the IRF4 transcription factor (62, 63). Moreover, the establishment of the concept that mouse *bona fide* DC constitute a separate hematopoietic lineage, and the discrimination between mouse CD11b$^+$ cDC and MoDC, were confirmed using mutant animals allowing to track natural precursor–progeny relationships *in vivo* through irreversible fluorescent tagging of all daughter cells of a given type of hematopoietic progenitor, based on Cre-mediated conditional activation

TABLE 1 | Different methodologies to define DC subsets with their advantages and drawbacks[a].

	Methodology				
	Cell surface phenotyping	**Ontogeny**	**Functional characterization**	**Molecular profiling**	
				At the population level	**At the single cell level**
Dependency on cell surface phenotyping	Not applicable	Yes but **methodology allows assessing the risk of cell type cross-contamination**	Yes, risk of bias Data quality heavily depends on rigor of the cell surface phenotyping procedure used to identify cell types	Yes, risk of bias Data quality heavily depends on rigor of the cell surface phenotyping procedure used to identify cell types. ***A posteriori*** **analyses can allow rigorously assessing the risk of cell type cross-contamination**	**No** ***Ab initio*** **identification of cell types without use of prior knowledge on their identity**
Experimental feasibility	**Good**	Difficult for most species except mouse	Depends on the species studied and the functions tested	**Good** Needs comparison with sister cell types and potential contaminants	Challenging both for data generation and data analysis. Commercial solutions exist for data generation but are expensive Needs to balance cost and sequencing depth. Data analysis still in a large part dependent upon knowledge from molecular profiling at the population level
Protocol standardization	**Achievable soon** but currently limited. Currently used markers defined fortuitously/empirically, generally unrelated to cell biology, and different between tissues, species, and laboratories	Difficult	Difficult The most subject to variations. Multiplicity of protocols depending on the functions tested, the tissues used and the species studied including its genetics, and even on the laboratories	**Good** **Routine technology for data generation** **Democratization of bioinformatics analyses**	Should happen upon technology maturation and democratization
Frequency of use	**Most frequent**	Mostly by specialists	**Frequent** Depending on the species studied and the functions tested	**Increasing frequency**	Very rare but high potential
Advancement of knowledge	The less informative	**Generally dichotomic information allowing relatively easy classification.** **Relevant to cell biology**	**Yes** **The most relevant for clinical and veterinary applications**	**Yes** **Generation of novel hypotheses on the ontogeny or functions of cells and their molecular regulation.** **Identification of conserved and biologically relevant cell surface markers.** **Identification of candidate molecular targets to manipulate cell functions**	**Yes** **Same advantages as molecular profiling at the population level.** **In addition,** **i) unbiased identification of cell types and associated transcriptomic signatures,** **ii) strong potential for identification of new cell types, iii) evaluation of intra-cell type heterogeneity, and** **iv) rigorous identification of cellular modules constituted of genes co-expressed in single cells and contributing to the same biological function**

[a]*Advantages are indicated in bold font and drawbacks in plain font.*

of a floxed reporter gene under the control of the constitutive Rosa26 promoter, an experimental strategy-coined fate mapping (64). Based on the important contribution of ontogenic studies for rigorous delineation of the identity of mouse DC subsets and of their lineage relationships, it has been proposed to use ontogeny as a primary methodology for the classification of mononuclear cell subsets in all species (57). Recent methodological progress has now made rigorous ontogenic studies applicable to human DC subsets, by using surrogate models of DC development from human $CD34^+$ hematopoietic progenitors, either *in vitro* (41, 65, 66) or *in vivo* in alymphoid mice (66–68). Such approaches have allowed demonstrating remarkable similarities in the ontogeny of mouse and human DC subsets. For example, knock-down experiments performed by transducing human $CD34^+$ hematopoietic progenitors with shRNA-expressing lentiviral vectors allowed to show that human pDC development critically depends on the transcription factor SPIB including *in vivo* in humanized mice (67), and that human $XCR1^+$ cDC development depends on the transcription factor BATF3 *in vitro* but not *in vivo* in humanized mice (68). Moreover, the pathway for the development of human pDC, $XCR1^+$ cDC, and $XCR1^-$ cDC was very recently demonstrated to be similar to that described for mouse DC subsets, with the identification of the human homologs to the mouse common DC progenitor and pre-cDC (66, 69). The role of candidate genes susceptible to affect DC development can even be assessed *in vivo* in humans in the rare cases where patients have been identified with primary immune deficiencies resulting from natural mutations in such genes (70). Strategies are being developed to actively search for human primary immunodeficiencies affecting DC development as experiments of nature allowing deciphering the molecular mechanisms regulating this biological process (71). However, ontogenic studies will often not be applicable in human for rigorous assessment of the identity of DC subsets, for example when studying a potentially known DC subset in a novel physiopathological context, including characterization of the DC subsets present in steady-state non-lymphoid tissues (50) or infiltrating tumors and their draining lymph nodes (72, 73) or isolated from infected/inflamed tissues. In addition, rigorous ontogenic studies will be very difficult to perform in many species, because (i) precursor/progeny relationships remain very difficult to evaluate *in vivo* through cell fate mapping or cell transfer experiments, (ii) *in vivo* analysis of cell subset development dependence on growth factors or transcription factors cannot be reasonably done due to operational and/or financial reasons, and (iii) *in vitro* models of *bona fide* DC development are currently lacking (74). Hence, the use of other methodologies will be necessary to prove DC subset identity in these various conditions.

Functional Characterization

Ideally, cell types should be defined based on the array of functions they can exert, because this definition links identity to function and is hence the most relevant to understand the functioning of the immune system and to harness the biology of DC subsets for improving health care of humans and of other species. In addition, cell type definitions based on their functional specialization could be the most universal across tissues and species. However, functional assays are often the hardest to perform experimentally and can be the most subject to variations depending on assays and experimental conditions. This is especially the case for assays aiming at comparing the ability of different DC subsets to activate T cells. If one aims at precisely comparing the cell-intrinsic ability of different DC subsets to process and present antigens, a number of potentially confounding factors must be taken into account to design the experiment in order to reduce the risk of inappropriate interpretation of results. Adequate steps must be taken to preserve the viability of DC subsets and control for it. This implies adding to each isolated DC subset the appropriate cytokines or growth factors necessary for their survival, for example GM-CSF for cDC and IL-3 for human pDC. For instance, sorted $XCR1^+$ cDC show a lower *ex vivo* survival as compared to $XCR1^-$ cDC in mice and sheep (75, 76). Sorting of DC subset by positive selections may affect DC subset responses due to antibody-mediated receptor stimulation (43, 77–79). This also implies including a positive control consisting in DC subsets pulsed with optimal epitopic peptides, to assess on antigen-specific T-cell priming by DC the impact of other factors than DC subset-intrinsic differences in antigen processing and presentation, not only differences in DC subset viability but also in delivery of co-stimulation or cytokine signals. In this regard, for a fair comparison between DC subsets, they should each be matured by stimulation with an appropriate adjuvant. PolyI:C is much more efficient than LPS for the activation of human $XCR1^+$ DC while it is the reverse for the activation of human MoDC. TLR7 or TLR9 ligands, but not TLR3 or TLR8 ligands, are potent activators of human pDC. Another layer of complexity is due to fundamental differences in the design of experiments in different species. While the gold standard for antigen processing and presentation assays in mice is the measurement of the activation of TcR-transgenic naïve T cells, this is not possible in other species where various surrogate readouts are used including antigen-specific re-activation of antigen-experienced T-cell clones or polyclonal T-cell lines or even proliferation of allogeneic T cells. It is known that significant differences exist in mice in the signals required for naïve T-cell priming, antigen-experienced T-cell re-activation, or allogeneic T-cell proliferation induction. Therefore, the same exact function is not fairly tested in different species. Furthermore, in species outside mice and humans, the use of epitopic peptide control requires to have accurate MHC typing and knowledge of the corresponding optimal peptides, which are generally unavailable. In addition, for accessibility reasons, the DC subsets used generally derived from different anatomic compartments depending on the species. For example, spleen DC subsets are often used in mice, blood, or tonsil DC in humans and lymph DC in sheep, which can further confound rigorous interpretation of the results when differences are observed between species. Finally, while inbred mice with defined sanitary status are generally used to limit the variability of the responses between individuals, this is not the case for other species including humans where the considerable heterogeneity in the genotypes, environments, and immune histories of individuals contribute to the strong variability of their responses (80). Hence, even for mouse experiments, there is a strong need for standardization of functional assays assessing the ability of DC subsets to process and present antigens and to functionally polarize T cells. Moreover, when attempting to compare DC

subset functional specialization across two species, efforts should be made to use comparable experimental designs in both species. Thus, while functional characterization is highly desirable when identifying a potentially new subset of DC or studying in a novel context a potentially known DC subset, the identity of DC subsets must first be studied through alternative approaches measuring cell type-specific parameters that are less strongly influenced by the tissue microenvironment and the genetic or immune history of populations, and for which experimental protocols are relatively well standardized.

Molecular Profiling at the Population Level

As the ontogeny and functions of cell types are instructed by specific gene expression modules, cell type identity can be defined by its molecular fingerprinting, including through gene expression profiling (81, 82). Homologous cell types between species can be defined as "those cells that evolved from the same precursor cell type in the last common ancestor" (82). This implies that homologous cell types must exhibit closer molecular fingerprints and gene expression programs than non-homologous cell types. Thus, it should be possible to decipher the identity of immune cell types of virtually all vertebrate species, by establishing their gene signatures and comparing them to the transcriptomic fingerprints of the well-characterized immune cell types of the mouse referent species. This is indeed an approach we pioneered to compare mouse spleen and human blood DC subsets (39) and later extended to comparison with sheep lymph cDC subsets (76), mouse DC subsets across tissues (40), as well as chicken spleen and pig skin mononuclear phagocyte subsets (83, 84). This approach allowed us to rigorously demonstrate for the first time to the best of our knowledge that human CD1c$^+$ cDC and CD141$^+$ cDC were homologous to mouse CD11b$^+$ cDC and CD8α^+ cDC, respectively (39, 85). This was later confirmed by us and others based on phenotypic, functional, and ontogeny studies (18, 37, 50, 65, 86). In addition, this approach permitted to show that cDC split into XCR1$^+$ and XCR1$^-$ subsets in migrating skin lymph DC in sheep, a species belonging to the Laurasiatherians, which is a mammalian order distant from the mouse and human Euarchontoglires (76). This approach also provided the first compelling evidence for existence of *bona fide* cDC and macrophages in chicken, showing that diversification in mononuclear phagocyte cell types appeared in a common ancestor to mammals and reptiles (83). Comparative transcriptomics also led to recognize CADM1 and SIRPα as surface molecules whose conserved expression throughout distant species can be used as a first phenotyping step to identify XCR1$^+$ and XCR1$^-$ cDC subsets in any mammal (76). Notably, CADM1 is a highly conserved molecule, presenting about 90% identity across mammalian orthologs, thus allowing using commercial anti-human CADM1 antibodies for cellular staining in distant species (76, 84). We found the *Xcr1* gene among genes specifically expressed in mouse spleen CD8α^+ DC when compared to a number of other immune cell types [see Supplementary Material "Additional file 5; gb-2008-9-1-r17-s5.xls" from Robbins et al. (39), specifically in the "CD8a_DC_gene_signature" established from our microarray data and confirmed from our own re-analysis of the microarray dataset independently generated by Dudziak et al. (87)]. Specific expression of the Xcr1 protein on mouse lymphoid tissue-resident CD8α^+ DC and its functions were first unveiled in the pioneering report from the group of Kroczek (36), who showed that CD8$^+$ T-cell cross-priming depends on their ability to secrete the Xcr1 ligand Xcl1 in experimental models where either the OVA coupled to an anti-CD205 Ab or OVA-expressing allogeneic pre-B cells are administrated *in vivo*. Xcr1 expression on CD8α^+ DCs was also found to be critical for the optimal induction of CD8$^+$ T-cell responses upon *Listeria monocytogenes* infection (18). Importantly, comparative transcriptomics revealed XCR1 as a specific and universal marker for XCR1$^+$ cDC across tissues and species. This was initially shown in human, mice, and sheep (18, 37, 76) and subsequently in non-human primates and pigs (18, 37, 38, 40, 52, 59, 60). Altogether, these studies were critical for the current proposal of cDC subset classification into XCR1$^+$ and XCR1$^-$ cDC (38, 40). Many other recent studies have demonstrated the power of gene expression profiling to determine with a high degree of certainty the identity of mononuclear phagocyte subsets in a tissue where they had not been rigorously studied before or to identify homologous subsets of mononuclear phagocytes across species (5, 6, 8, 9, 50, 88–90). Importantly, standardized protocols for generation and analysis of gene expression data are routinely performed in many laboratories, platforms, or commercial companies in many countries. The corresponding costs have strongly decreased over the last decade and continue to go down. Hence, gene expression profiling at the population level is a very robust and reproducible methodology that is feasible in virtually all species where tools are available or can be developed to phenotypically identify and purify candidate cell subsets. However, potentially confounding factors must be taken into account to design experiments in order to reduce the risk of inappropriate interpretation of results (**Figure 3**). First and foremost, great care and rigor must be exerted in designing the experimental sampling protocol for cell subset purification, inasmuch as minor contamination by another cell type can dramatically impact the gene expression profile obtained. Hence, it is critical to carefully design the marker combination used to purify the different cell populations to be studied, and to control cell purity prior to the generation of the gene expression data. Second, to allow proper analysis of the gene expression profiles of the targeted cell type, appropriate cell type controls must be included, encompassing sister cell types as well as cell types that could be potential contaminants due to their expression of several of the markers used for positive selection of the targeted cell type. These controls are critical to allow assessing the risk of contamination by another cell type (49).

Molecular Profiling at the Single Cell Level

Recent technological advances now allow performing high throughput RNA sequencing at single cell levels with high sensitivity and processivity. Transcriptomic analyses at the single cell level could solve most of the issues raised in the previous section for molecular profiling at the population level. Indeed, because it alleviates the necessity to purify cells on imperfect and potentially confounding phenotypic marker combinations, analysis at the single cell level should allow unbiased identification of potentially all cell types and their associated transcriptomic signatures. It also solves the issue of cross-contamination between cell types, since the identity of each single cell is established *a*

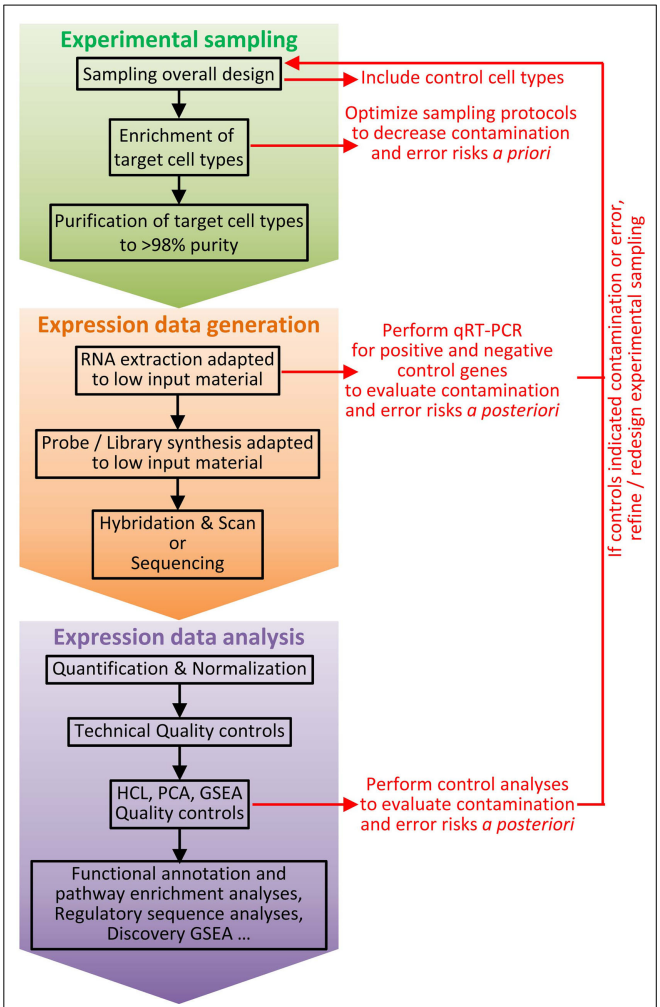

FIGURE 3 | Workflow for cell type identification by molecular profiling at the population level. Molecular profiling at the population level can be very informative for cell type identification. However, inappropriate interpretation can occur if confounding factors are not taken into account. Hence, it is critical to carefully design experiments and to establish a rigorous workflow, including a number of key control samples and quality check procedures. The experimental sampling protocol must be optimized to decrease *a priori* the risk of cross-contamination between cell types or of error resulting in selection of another cell type than the one wanted. Purity of cell types must be assessed immediately after sampling (e.g., by flow cytometry). Positive and negative cell type controls must be included, such as sister cell types and potential contaminant populations. Once molecular expression data have been obtained, after technical quality has been validated by classical controls, additional specific quality controls must be performed to *a posteriori* ensure of lack of cross-contamination between cell subsets or to evaluate the risk of misinterpretation of the results. HCL, hierarchical clustering; PCA, principal component analysis; GSEA, Gene Set Enrichment Analysis.

posteriori based on the analysis of its gene expression program. In addition, the generation of gene expression data for many individual cells of the same type should increase statistical power to define genes co-expressed at the single cell level and to define cell type-specific transcriptomic modules (81). As a proof-of-principle, single cell gene expression profiling recently allowed the unbiased and *de novo* identification of the different cell types of spleen (91) and central nervous system (92, 93) via the description of their molecular identity, starting from the bulk population of all the cells that could be extracted from the organ without any prior enrichment procedure. However, molecular profiling at the single cell level cannot be used without prior phenotype-based enrichment for very rare cell types, and it is difficult to apply to species in which genome has not yet been completely assembled. To obtain complete information, including on functionally important genes for which few mRNA are expressed per cell, it is necessary to sequence at a sufficient depth of about one million reads per cell, which today still represents a very high cost when multiplied by the number of individual cells and conditions. This is all the more the case since, likewise for molecular profiling at population level, correct interpretation of the data requires that sister cell types as well as cell types that could be potential contaminants are included in the experimental design. Moreover, the technology for single cell RNA sequencing is not yet democratized, since it is challenging both for sample preparation and for data analysis. For standardization of high quality sample preparation, commercial solutions exist but are very expensive. For data analysis, there is no consensus yet on how the data should be mathematically modeled for adequate removal of background signal and for discrimination of false negative signal due to sampling bias in the pool of the cell mRNA as opposed to true lack of gene expression. In addition, the interpretation of the RNA-seq data on single cells is still largely based on the transcriptomic/molecular identity of cell types that are deduced from microarray analysis of purified cell pools (91). Hence, molecular profiling at the population level currently represents a more sustainable strategy for most laboratories.

Recent Advances Brought by Comparative Transcriptomics at the Population Level for Defining the Identity and Functions of Mononuclear Phagocyte Subsets and Their Molecular Regulation

In this section, we will review major advances brought forward by comparative transcriptomics at the population level for defining the identity and functions of mononuclear phagocyte subsets and their molecular regulation.

Gene expression profiling of cell types with apparent ambiguous phenotype or functions allowed to rigorously establish their identity, which could be achieved properly strictly contingent to their comparison with all candidate sister cell subsets as well as more distantly related cell types. Hence, we and others showed that human blood Lineage$^-$CD16$^+$ cells are non-classical monocytes (39, 88) and not DC as was sometimes claimed (94–96). Similarly, analysis of human skin CD14$^+$ cell expression of the transcriptomic fingerprints independently established for cDC, monocytes, and macrophages provided critical evidence that these cells are monocyte-derived macrophages (5) while they were previously designated as DC (4). Transcriptomic analyses were also instrumental to demonstrate the homology of this human dermal cell type with the murine CD11b$^+$Ly6C$^-$CD64^{lo-hi} (6) and pig CD163$^+$ (84) skin subsets. We were also able to show that cell populations claimed to correspond to novel cell types actually corresponded to a distinct differentiation or activation state of an already known cell type, for example establishing that the

so-called interferon killer DC correspond to a particular activation state of NK cells (39). Furthermore, we showed that, upon many types of *in vivo* or *in vitro* stimulation, human and murine pDC and cDC undergo a remodeling of their gene expression program related to their plasticity, including induction of NFκB and IFN target genes, but still keep the canonical gene expression associated to their subset identity (41, 97). In particular, contrary to what other researchers hypothesized (98), gene expression profiling showed that activated pDC are not undergoing a cell fate conversion into a novel type of cDC (97).

Gene expression profiling also allowed aligning subsets of mononuclear cells across tissues (6, 8, 9, 40, 55, 99), establishing cell type homologies across species (5, 39, 50, 76, 83–85, 88, 89, 100), and rigorously examining the proximity of *in vitro*-derived subsets of mononuclear cells with those naturally existing *in vivo* (39, 41, 66, 101). These studies allowed significantly advancing the ontogeny and functional characterization of mononuclear phagocyte subsets based on the novel hypotheses that can be inferred from the analysis of the gene expression programs of the cells and from their comparison with other well-characterized cell types.

The study of the functional specialization of human DC subsets was strongly boosted by the demonstration of their transcriptomic homologies with mouse DC subsets (39, 85) which was recognized as a major breakthrough in the field (37, 53, 102–104) and acknowledged to have been impossible to draw from studies based on a limited set of molecular markers (105). In particular, this led to test whether human XCR1$^+$ cDC could be more efficient for cross-presentation than other human DC subsets. Even though the extent to which human XCR1$^+$ cDC are more efficient for cross-presentation than other human DC subsets is debated, the results from the functional studies performed independently by many teams concurrently demonstrate that these cells excel at cross-presentation of cell-associated antigens (18, 19, 37, 41, 86, 106) and of particulate antigens delivered through FcγR, through late endosomal targeting (21, 107) or upon polyI:C stimulation (18, 41, 86, 108). In addition, in sheep, the skin lymph migrating XCR1$^+$ cDC spontaneously displayed a higher efficiency of soluble antigen-presentation to specific CD8$^+$ T cells, as compared to XCR1$^-$ cDC (76).

Based on the demonstration of the striking transcriptomic similarities between mouse and human subsets of mononuclear cells, and on knowledge on the ontogeny of these cells in the mouse (109, 110), we proposed that, similar to their mouse counterparts, human pDC and cDC constitute a specific family of cells within the hematopoietic tree, should derive from a common progenitor with a DC-restricted differentiation potential, and could be derived *in vitro* from human CD34$^+$ progenitor cells in part under the instruction of the FLT3-L growth factor (39, 85), all of which was later confirmed experimentally (41, 65, 66, 69, 111, 112).

Very importantly, comparative genomics of immune cell subsets yielded conserved transcriptomic fingerprints for each of these cell types (39), a novel knowledge which considerably accelerated the deciphering of the molecular mechanisms regulating the development and functions of leukocytes as reviewed in **Table 2** (18, 36, 59, 100, 113–127). Finally, this approach uniquely allowed identifying conserved and biologically relevant cell surface markers for each subset of mononuclear cells which could enable considerably simplifying the nomenclature for DC subsets by using the same name and similar marker combinations to identify homologous cell types irrespective of their tissues and species of origin (55–57).

Conclusion and Perspectives

While it might be the case in the future for single cell RNA-seq, currently no single method is sufficient to allow the best possible classification of DC. Hence, ideally, all available methods (cell surface phenotyping, gene expression profiling, functional analyses, and ontogeny) should be combined together to define DC subset identity. However, such a combination of approaches cannot be used to define cell subsets in many instances due to technical, financial, or ethical limitations. Taking these limitations into consideration, the data reviewed here show that comparative transcriptomics at the population level is currently the most robust

TABLE 2 | Genes which selective expression pattern in immune cell types was uncovered through comparative genomics and which functions in these cells were deciphered later.

Transcriptomic signature[a]	Gene symbol (alias)	Function
pDC	*PACSIN1*	Necessary for pDC production of type I interferons upon TLR7/9 stimulation (115)
	RUNX2	Necessary for terminal differentiation of pDC in, and their egress from, bone marrow (114)
	TCF4 (*E2-2*)	Master transcription factor instructing pDC development and functions (113)
	BCL11A	Necessary for pDC development (116, 117)
cDC	*ZBTB46* (*BTBD4*)	Transcription factor that appears to be a specific marker of the cDC and endothelial lineages and which limits spontaneous cDC maturation (118, 119, 128)
	BATF3 (*9130211I03Rik*)	Transcription factor which can be critical for development of XCR1$^+$ cDC depending on the context (121)
cDC above pDC	*BCL6*	Promotes the development of XCR1$^+$ cDC (99, 120)
XCR1$^+$ cDC above XCR1$^-$ cDC and pDC	*TLR3*	TLR3 triggering induces a very strong activation of mouse and human XCR1$^+$ cDC including a uniquely high production of IFN-β and type III IFN (41, 100, 129, 130)
	RAB11A	Functionally promotes cross-presentation by storing MHC class I in a unique endosomal recycling compartment (122)
Mouse XCR1$^+$ cDC	*XCR1*	Likely promotes efficient interactions between XCR1$^+$ cDC and NK cells or CD8$^+$ T cells (18, 36)
Pan-T cells	*THEMIS* (*E430004N04Rik*)	Sets the signal threshold for positive and negative selection of developing T cells in the thymus (124–127)
	BCL11B	Regulates critical aspects of the development, functions, and homeostasis of T cells (123)

[a] *Transcriptomic signatures conserved between mouse and human unless specified otherwise, first reported in Robbins et al. (39), and encompassing the genes listed in this table.*

and feasible way to define the identity of cell types. Indeed, because the ontogeny and functions of cell types are instructed by specific gene expression modules, cell type identity can be defined in a universal and unbiased way by its molecular fingerprinting, including through gene expression profiling (81). However, due to its dependency on pre-selection of cell populations based on their expression patterns of a few cell surface molecules, gene expression profiling at the cell population level is imperfect and may require iterative steps of refined cell type isolation and gene expression profiling as illustrated in **Figure 3**. Hence, it is all the more important that each step of the procedure is performed and rigorously quality controlled according to the best standards in the field.

Cell purity is fundamental. It is important to design a sampling method specific for each study, through identification of the most robust criteria available in the current state of the art for purification of the target cell type based on phenotypic, morphologic, or anatomical characteristics. Cell enrichment is necessary for rare cell types among bulk populations. It relies on the depletion of other populations (MACS or EasySep™ for instance). The marker combination for negative selection must not unwillingly remove a population of interest. For instance, some antibody cocktails for human DC enrichment use anti-CD16 monoclonal antibodies, so as to deplete NK cells, but this should be proscribed for the study of non-classical, $CD16^+$ monocytes. Positive selection by magnetic or flow cytometry sorting is most often required after cell enrichment. Antibody labeling must be clear-cut, with separate peaks and/or selection of the events with the highest labeling and the lowest potential contamination by other populations. This selection implies the use of marker combinations specific for the population of interest, since specific markers are rarely available. XCR1 is a rare instance of a conserved marker so far only expressed on a discrete DC population. To the best of our knowledge, reliable commercial reagent are available for XCR1 staining only for mouse and rat, but XCR1 staining can also be achieved with fluorescently labeled recombinant XCL1 (40, 41, 52), a strategy that is amenable to many species in which XCL1 sequence is known. CLEC4C alias BDCA2 and LILRA4 alias ILT7 are specific markers for human pDC, but their engagement induces inhibitory signals which for instance reduce pDC production of type I interferons after stimulation (43, 77–79, 131). Although selectively expressed at high levels on human pDC in the blood or lymphoid organs under steady-state conditions, NRP1 alias BDCA4 can be induced on activated cDC and is also expressed on other cell types including neurons, endothelial cells, and tumor cells (132, 133). CD123 is a good marker to help identifying pDC in non-human primates, but it also labels mastocytes which are present in the blood or in lymphoid organs (134). Cell purity must be controlled in each experiment, by flow cytometry re-analysis just after sorting, and as one of the first step of transcriptomic analysis by examining the expression of negative and positive control genes (expression of genes that should be expressed only on other populations including potential contaminants, and expression of genes characteristic for the population of interest including but not restricted to genes coding for the molecules used for positive selection) (**Figure 3**).

The quality and quantity of mRNA must be adequate, even when cell numbers are low. RNA extraction kits adapted to low cell number samples may be required. mRNA quality must be controlled by electrophoresis. A linear amplification protocol must be used, that has been validated for yielding results from low input RNA showing a strong correlation with the results obtained with higher RNA input and a classical amplification procedure.

For bioinformatics analyses, the dataset must include sister cell types as well as the cell types the most likely to contaminate the cell type of interest, or at least be compatible for integrative analysis with a reference dataset including these control populations. Several independent methods for data analysis should be used, to ensure robustness of interpretation. Beyond relative classification of the cell types of the dataset by classical approaches computing the overall distance between their gene expression programs as performed by hierarchical clustering or principal component analysis, the identity of cell types can also be reliably inferred from the analysis of their relative expression of robust cell type-specific gene signatures established from re-analysis of public gene chip databases and/or from published articles.

Novel advances are being brought through molecular profiling of subsets of mononuclear cells. In addition to steady-state conditions, populations can be analyzed after stimulation to identify the specific activation pathways elicited in pure cell populations or upon interaction between different cell types (41, 97, 135–137). In addition to unbiased analysis of the cellular composition of different organs (91, 93), transcriptomic profiling at the single cell level will allow studying heterogeneity in gene expression within one cell type with the hope to link it to functional heterogeneity (138) and eventually with the former history/epigenetic imprinting of each cell. Comparative transcriptomic studies allowed us and others to identify in humans, non-human primates, pig, sheep, and chicken cDC subsets homologous to those well described in mice (5, 18, 39, 50, 52, 62, 76, 83–85). These studies suggest that similar cDC subsets already existed in the last common ancestor of birds and mammals. Conserved gene modules appear during evolution to elicit new functions (81, 82). For instance, regarding T helper lineage diversification during evolution, contrary to bony fishes, the elephant shark, a cartilaginous fish, has been reported to lack genes encoding for critical transcription factors or cytokines instructing the development or involved in the functions of Th2, Th17, and Treg cells, such as RORC and FOXP3, IL-4, IL-21, IL-23, and IL-2 (139). This suggests that the genes required for the development of the different T helper lineages might have appeared progressively as modules during evolution starting in bony fishes and with late development of the Treg and Th17 lineages (81). Comparative genomics of mononuclear phagocyte subsets and single cell gene expression profiling will critically help identifying novel gene modules and their associated immune functions. In pDC, evolutionarily conserved co-expression of *TCF4*, *RUNX2*, *TLR7*, *TLR9*, *UNC93B1*, *MYD88*, *IRAK4*, *IRF7*, and *PACSIN1* might represent part of a gene module instructing the functional specialization of this cell type in high level production of type I interferon in response to sensing of oligonucleotide sequences of viral or autologous origin. In $XCR1^+$ cDC, evolutionarily conserved co-expression of *CLEC9A*, *SYK*, *RAB11A*, *RAB7B*, *SEPT3*, *SNX22*, *TLR3*, *CADM1*, and *XCR1* might represent part of a gene module instructing the functional specialization of this cell type in $CD8^+$ T-cell activation and specifically in cross-presentation of cell-associated antigens.

In any case, the discovery of the sets of genes that are tightly co-expressed in DC subsets across various tissues and species, not only at the population level but also at the single cell level, should allow identifying the gene modules instructing DC subset functions. Characterization of the members of these gene modules which role in DC is unknown yet should strongly contribute to increase our knowledge on DC subset functional specialization and their molecular regulation. Of note, not all of these gene modules might harbor the same differential pattern of expression between DC subsets in different animal species. Some functions have gained or lost expression in specific cell subsets in some species which should correlate with similar changes in the expression patterns of the corresponding gene modules. For instance, IL-12 is produced both by pDC and cDC in mice, but only by cDC in humans, while antigen cross-presentation appears to be more strongly associated with XCR1$^+$ cDC in mice than in humans (18, 19, 22). Isolation and comparison of mononuclear phagocyte subsets from homologous organs in different species may help understand how the anatomical compartmentalization of these cells is established and affects their functions, including local interaction with specific cell types and chemokines. Dating when during evolution pDC as well as classical and non-classical monocyte subsets appeared, and in which anatomical compartments they reside in the species the most distant to humans and mice, may give novel insights into the core functions of these populations.

In vivo manipulation of DC can promote and orient immune responses based on the intrinsic functional properties of the DC subset targeted and can be advantageously used for prophylactic vaccination or immunotherapy against cancer or infections. This strategy can benefit from the knowledge gained from the expression profiling of DC subsets and their alignment across species. Notably, based on their homology with mouse XCR1$^+$ cDC, human XCR1$^+$ cDC can be considered as a promising target when cross-presentation is desirable, in particular for fighting cancer or infections by intracellular pathogens (23, 24, 29, 72, 73, 140–143). Moreover, because it is specifically expressed in XCR1$^+$ cDC in a conserved manner in evolution, and it has been successfully used for *in vivo* delivery of antigens specifically to XCR1$^+$ cDC to vaccinate mice (23, 24), XCR1 can be considered for a universal DC targeting strategy in potentially all vertebrate species. Interestingly, the targeting of XCR1 can be achieved with targeting units composed of recombinant XCL1 fused to protective antigens in the form of vaccibodies (24), a strategy that is amenable to many species in which the XCL1 sequence is known. Although more broadly expressed in the DC lineage at least in mice, CLEC9A is also an interesting target since it directly promotes cross-presentation of the material it binds, probably by delivering it into appropriate endosomes (144, 145), and because it is selectively expressed to high levels on XCR1$^+$ cDC in humans, sheep, and mice (25, 32, 76, 146) although it may not be the case in some other species such as pig. Arguments in favor or against the targeting of XCR1$^+$ cDC in the clinic are summarized in **Table 3**. The identification of XCR1$^+$ cDC in companion and sport animals, and in animals of the agro-economy, such as ruminants, pigs, poultry, and fishes, will allow designing better vaccines to protect

TABLE 3 | The PROs and CONs for *in vivo* targeting of XCR1$^+$ cDC[a].

	PROs	CONs
Cross-presentation efficiency	Higher for blood and skin XCR1$^+$ cDC, especially for cell-associated antigens	Disputed for XCR1$^+$ cDC from secondary lymphoid organs (19, 22) depending on intracellular compartment of antigen delivery (21)
Anatomical localization	Present in lymphoid and non-lymphoid tissues, enabling subcutaneous, intradermal, or oral vaccination	Low efficiency of human XCR1$^+$ cDC for induction of mucosa-homing CD8$^+$ T cells (151)?
Frequency	Few cells can mediate important functions *in vivo*. Quality matters more than quantity	Very few numbers of XCR1$^+$ cDC in most tissues
Specificity of targeting	Very specific expression of XCR1 as opposed to the broader expression of CD141, DEC205, and CLEC9A. Precise targeting and better pharmacodynamics	Too specific, limiting biological effect to just one DC subset, may not induce strong enough or broad enough immune responses
Responsiveness to adjuvants	Very good responsiveness to PolyI:C. PolyI:C is a very potent adjuvant for the induction of strong, polyfunctional CD8$^+$ T-cell responses which might result in part from TLR3 triggering in XCR1$^+$ cDC	PolyI:C may primarily work by activating other targets, i.e., non-immune cells expressing TLR3 or cells activated through MDA5
Proof of concept achieved in mice	XCR1$^+$ cDC are critical for anti-tumoral responses in mice (72, 121, 152, 153). XCR1 targeting works in mice (23, 24). XCR1 bio-equivalency in human, macaques, mouse, pig, and sheep, same gene expression pattern and biological function. Hence, higher probability of translation to human of mechanistic studies in animals	Many previous failures of mouse to human translation
In vitro model	Ability to generate *in vitro* and manipulate *bona fide* human XCR1$^+$ cDC from CD34$^+$ cord blood progenitors (41, 65, 66, 69, 111, 112)	
Cytokine production	XCR1$^+$ cDC can produce IL-12 but maybe optimal conditions to induce this function remain to be identified (50, 65, 66, 143). Mouse and human XCR1$^+$ cDC are high producers of beta and type III interferons upon PolyI:C stimulation (41, 100, 129, 130)	Human XCR1$^+$ cDC are very poor producers of IL-12 (70, 108)
Clinical data	Gene expression profiling of human tumors suggest that infiltration by XCR1$^+$ cDC but not other myeloid cells is of good prognosis both in mice and humans (72)	Formal measurements of XCR1$^+$ cDC infiltration in human tumors and of its beneficial role for disease control remain to be established

[a]*More details and bibliographical references can be found in the main text of this review.*

TABLE 4 | Practical guidelines for consistent definition of DC subsets across mouse and human tissues with potential applicability to other mammals.

Characterization	XCR1$^-$ cDC2		XCR1$^+$ cDC1		pDC	
	High or positive	**Negative or low**	**High or positive**	**Negative or low**	**High or positive**	**Negative or low**
Conserved phenotype	CD11c^{high} MHC-II^{high} FLT3$^+$ SIRPα^+	CD3$^-$ CD19$^-$ CD14$^{-/low}$ CD206$^{-/low}$ CD123$^-$	CD11c$^{low\text{-}to\text{-}high}$ MHC-II^{high} FLT3$^+$ XCR1$^+$ CADM1$^+$	CD3$^-$ CD19$^-$ CD14$^{-/low}$ CD206$^{-/low}$ CD123$^-$	MHC-II^{int} FLT3$^+$	CD3$^-$ CD19$^-$ CD14$^{-/low}$ CD206$^{-/low}$ CD19$^-$
Critical species-specific phenotypic markers					Mouse: Siglec-H or Ccr9 Human: CD123 and CLEC4C (BDCA2) or ILT7 (LILRA4)	
Hallmark genes (18, 37, 39)	*FLT3* *TLR8* *ZBTB46* ***IRF4***[a]	*XCR1* *RAB7B* *GCET2* *TLR4* *IRF8* *TCF4* *RUNX2* *SPIB*	*FLT3* *XCR1* *CADM1* *TLR3* *RAB7B* *GCET2* *ZBTB46* ***IRF8*** ***BATF3***	*TLR4* *TLR7* *IRF4* *TCF4* *RUNX2* *SPIB*	*FLT3* *TLR7* *TLR9* *PACSIN1* ***IRF8*** ***TCF4*** ***RUNX2*** ***SPIB*** ***BCL11A***	*XCR1* *CADM1* *TLR3* *TLR8* *RAB7B* *GCET2* *ZBTB46* *BATF3* *CADM1*
Hallmark cytokine production	IL-23 production? (62)		Type III interferon production upon TLR3 triggering (41, 100, 129, 130)		Production of type I and III interferons in response to TLR7/9 triggering	
Hallmark antigen-presentation functions	High efficiency for CD4$^+$ T-cell activation		High efficiency for CD8$^+$ T-cell activation, in particular through cross-presentation of cell-associated antigens			

[a]*Master transcription factors critical for cell subset development are indicated in bold font.*

them against infections in order to ameliorate animal welfare and to prevent pandemics causing severe economic losses. It will also contribute to a global public health strategy because some of these animal species as well as wild animals are targets or reservoirs for major zoonotic pathogens. The identification of XCR1$^+$ cDC in rhesus macaques and in pigs opens the way to preclinical vaccination studies in these species which are close to humans. Vaccibodies based on XCL1 dimers coupled to influenza or SIV proteins are planned to be used for vaccination of pigs or rhesus macaques, respectively, and induction of immune responses and protection against infection. pDC targeting could also be considered as an interesting alternative for vaccination against viruses or tumors (20, 147, 148), or for the induction of cross-tolerance to treat autoimmune diseases or food allergies (149, 150).

A synthetic list of phenotypic, transcriptomic, and functional hallmarks which have already allowed conserved identification of different DC and monocyte subsets in humans and mice is presented in **Table 4**. The present Special Issue and future workshop on DC nomenclature will help reach a consensus panel for practical definition of the populations, in order to integrate faster the huge, but scattered knowledge accumulated by different laboratories in different cell types, species, and organs.

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References

1. Cheong C, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP, et al. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* (2010) **143**(3):416–29. doi:10.1016/j.cell.2010.09.039
2. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* (2013) **38**(2):322–35. doi:10.1016/j.immuni.2012.10.016
3. Langlet C, Tamoutounour S, Henri S, Luche H, Ardouin L, Gregoire C, et al. CD64 expression distinguishes monocyte-derived and conventional dendritic cells and reveals their distinct role during intramuscular immunization. *J Immunol* (2012) **188**(4):1751–60. doi:10.4049/jimmunol.1102744

4. Klechevsky E, Morita R, Liu M, Cao Y, Coquery S, Thompson-Snipes L, et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity* (2008) **29**(3):497–510. doi:10.1016/j.immuni.2008.07.013
5. McGovern N, Schlitzer A, Gunawan M, Jardine L, Shin A, Poyner E, et al. Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages. *Immunity* (2014) **41**(3):465–77. doi:10.1016/j.immuni.2014.08.006
6. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* (2013) **39**(5):925–38. doi:10.1016/j.immuni.2013.10.004
7. Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med* (2012) **209**(6):1167–81. doi:10.1084/jem.20120340
8. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**(11):1118–28. doi:10.1038/ni.2419
9. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* (2012) **13**(9):888–99. doi:10.1038/ni.2370
10. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-derived interleukin-34 controls the development and maintenance of Langerhans cells and the maintenance of microglia. *Immunity* (2012) **37**(6):1050–60. doi:10.1016/j.immuni.2012.11.001
11. Perdiguero EG, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2014) **518**(7540):547–51. doi:10.1038/nature13989
12. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* (2012) **336**(6077):86–90. doi:10.1126/science.1219179
13. Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat Immunol* (2012) **13**(8):753–60. doi:10.1038/ni.2360
14. Dadaglio G, Fayolle C, Zhang X, Ryffel B, Oberkampf M, Felix T, et al. Antigen targeting to CD11b+ dendritic cells in association with TLR4/TRIF signaling promotes strong CD8+ T cell responses. *J Immunol* (2014) **193**(4):1787–98. doi:10.4049/jimmunol.1302974
15. Desch AN, Gibbings SL, Clambey ET, Janssen WJ, Slansky JE, Kedl RM, et al. Dendritic cell subsets require cis-activation for cytotoxic CD8 T-cell induction. *Nat Commun* (2014) **5**:4674. doi:10.1038/ncomms5674
16. Mouries J, Moron G, Schlecht G, Escriou N, Dadaglio G, Leclerc C. Plasmacytoid dendritic cells efficiently cross-prime naive T cells in vivo after TLR activation. *Blood* (2008) **112**(9):3713–22. doi:10.1182/blood-2008-03-146290
17. Ballesteros-Tato A, Leon B, Lund FE, Randall TD. Temporal changes in dendritic cell subsets, cross-priming and costimulation via CD70 control CD8(+) T cell responses to influenza. *Nat Immunol* (2010) **11**(3):216–24. doi:10.1038/ni.1838
18. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, Ventre E, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**(6):1283–92. doi:10.1084/jem.20100223
19. Segura E, Durand M, Amigorena S. Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. *J Exp Med* (2013) **210**(5):1035–47. doi:10.1084/jem.20121103
20. Hoeffel G, Ripoche AC, Matheoud D, Nascimbeni M, Escriou N, Lebon P, et al. Antigen crosspresentation by human plasmacytoid dendritic cells. *Immunity* (2007) **27**(3):481–92. doi:10.1016/j.immuni.2007.07.021
21. Cohn L, Chatterjee B, Esselborn F, Smed-Sorensen A, Nakamura N, Chalouni C, et al. Antigen delivery to early endosomes eliminates the superiority of human blood BDCA3+ dendritic cells at cross presentation. *J Exp Med* (2013) **210**(5):1049–63. doi:10.1084/jem.20121251
22. Mittag D, Proietto AI, Loudovaris T, Mannering SI, Vremec D, Shortman K, et al. Human dendritic cell subsets from spleen and blood are similar in phenotype and function but modified by donor health status. *J Immunol* (2011) **186**(11):6207–17. doi:10.4049/jimmunol.1002632
23. Hartung E, Becker M, Bachem A, Reeg N, Jakel A, Hutloff A, et al. Induction of potent CD8 T cell cytotoxicity by specific targeting of antigen to cross-presenting dendritic cells in vivo via murine or human XCR1. *J Immunol* (2015) **194**(3):1069–79. doi:10.4049/jimmunol.1401903
24. Fossum E, Grodeland G, Terhorst D, Tveita AA, Vikse E, Mjaaland S, et al. Vaccine molecules targeting Xcr1 on cross-presenting DCs induce protective CD8(+) T-cell responses against influenza virus. *Eur J Immunol* (2015) **45**(2):624–35. doi:10.1002/eji.201445080
25. Caminschi I, Proietto AI, Ahmet F, Kitsoulis S, Shin Teh J, Lo JC, et al. The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. *Blood* (2008) **112**(8):3264–73. doi:10.1182/blood-2008-05-155176
26. Caminschi I, Vremec D, Ahmet F, Lahoud MH, Villadangos JA, Murphy KM, et al. Antibody responses initiated by Clec9A-bearing dendritic cells in normal and Batf3(-/-) mice. *Mol Immunol* (2012) **50**(1–2):9–17. doi:10.1016/j.molimm.2011.11.008
27. Idoyaga J, Lubkin A, Fiorese C, Lahoud MH, Caminschi I, Huang Y, et al. Comparable T helper 1 (Th1) and CD8 T-cell immunity by targeting HIV gag p24 to CD8 dendritic cells within antibodies to Langerin, DEC205, and Clec9A. *Proc Natl Acad Sci U S A* (2011) **108**(6):2384–9. doi:10.1073/pnas.1019547108
28. Lahoud MH, Ahmet F, Kitsoulis S, Wan SS, Vremec D, Lee CN, et al. Targeting antigen to mouse dendritic cells via Clec9A induces potent CD4 T cell responses biased toward a follicular helper phenotype. *J Immunol* (2011) **187**(2):842–50. doi:10.4049/jimmunol.1101176
29. Li J, Ahmet F, Sullivan LC, Brooks AG, Kent SJ, De Rose R, et al. Antibodies targeting Clec9A promote strong humoral immunity without adjuvant in mice and non-human primates. *Eur J Immunol* (2014) **45**(3):854–64. doi:10.1002/eji.201445127
30. Park HY, Light A, Lahoud MH, Caminschi I, Tarlinton DM, Shortman K. Evolution of B cell responses to Clec9A-targeted antigen. *J Immunol* (2013) **191**(10):4919–25. doi:10.4049/jimmunol.1301947
31. Joffre OP, Sancho D, Zelenay S, Keller AM, Reis e Sousa C. Efficient and versatile manipulation of the peripheral CD4+ T-cell compartment by antigen targeting to DNGR-1/CLEC9A. *Eur J Immunol* (2010) **40**(5):1255–65. doi:10.1002/eji.201040419
32. Sancho D, Mourao-Sa D, Joffre OP, Schulz O, Rogers NC, Pennington DJ, et al. Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. *J Clin Invest* (2008) **118**(6):2098–110. doi:10.1172/JCI34584
33. Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S, Soares H, et al. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* (2004) **199**(6):815–24. doi:10.1084/jem.20032220
34. Nchinda G, Kuroiwa J, Oks M, Trumpfheller C, Park CG, Huang Y, et al. The efficacy of DNA vaccination is enhanced in mice by targeting the encoded protein to dendritic cells. *J Clin Invest* (2008) **118**(4):1427–36. doi:10.1172/JCI34224
35. Do Y, Didierlaurent AM, Ryu S, Koh H, Park CG, Park S, et al. Induction of pulmonary mucosal immune responses with a protein vaccine targeted to the DEC-205/CD205 receptor. *Vaccine* (2012) **30**(45):6359–67. doi:10.1016/j.vaccine.2012.08.051
36. Dorner BG, Dorner MB, Zhou X, Opitz C, Mora A, Guttler S, et al. Selective expression of the chemokine receptor XCR1 on cross-presenting dendritic cells determines cooperation with CD8+ T cells. *Immunity* (2009) **31**(5):823–33. doi:10.1016/j.immuni.2009.08.027
37. Bachem A, Guttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J Exp Med* (2010) **207**(6):1273–81. doi:10.1084/jem.20100348
38. Gurka S, Hartung E, Becker M, Kroczek RA. Mouse conventional dendritic cells can be universally classified based on the mutually exclusive expression of XCR1 and SIRPα. *Front Immunol* (2015) **6**:35. doi:10.3389/fimmu.2015.00035
39. Robbins SH, Walzer T, Dembele D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* (2008) **9**(1):R17. doi:10.1186/gb-2008-9-1-r17

40. Crozat K, Tamoutounour S, Vu Manh TP, Fossum E, Luche H, Ardouin L, et al. Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the CD8alpha+ type. *J Immunol* (2011) **187**(9):4411–5. doi:10.4049/jimmunol.1101717
41. Balan S, Ollion V, Colletti N, Chelbi R, Montanana-Sanchis F, Liu H, et al. Human XCR1+ dendritic cells derived in vitro from CD34+ progenitors closely resemble blood dendritic cells, including their adjuvant responsiveness, contrary to monocyte-derived dendritic cells. *J Immunol* (2014) **193**(4):1622–35. doi:10.4049/jimmunol.1401243
42. Yamazaki C, Miyamoto R, Hoshino K, Fukuda Y, Sasaki I, Saito M, et al. Conservation of a chemokine system, XCR1 and its ligand, XCL1, between human and mice. *Biochem Biophys Res Commun* (2010) **397**(4):756–61. doi:10.1016/j.bbrc.2010.06.029
43. Dzionek A, Sohma Y, Nagafune J, Cella M, Colonna M, Facchetti F, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med* (2001) **194**(12):1823–34. doi:10.1084/jem.194.12.1823
44. Rissoan MC, Duhen T, Bridon JM, Bendriss-Vermare N, Peronne C, de Saint Vis B, et al. Subtractive hybridization reveals the expression of immunoglobulin-like transcript 7, Eph-B1, granzyme B, and 3 novel transcripts in human plasmacytoid dendritic cells. *Blood* (2002) **100**(9):3295–303. doi:10.1182/blood-2002-02-0638
45. Cao W, Rosen DB, Ito T, Bover L, Bao M, Watanabe G, et al. Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits toll-like receptor-induced interferon production. *J Exp Med* (2006) **203**(6):1399–405. doi:10.1084/jem.20052454
46. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* (2000) **165**(11):6037–46. doi:10.4049/jimmunol.165.11.6037
47. Dalod M, Salazar-Mather TP, Malmgaard L, Lewis C, Asselin-Paturel C, Briere F, et al. Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. *J Exp Med* (2002) **195**(4):517–28. doi:10.1084/jem.20011672
48. Bar-On L, Birnberg T, Lewis KL, Edelson BT, Bruder D, Hildner K, et al. CX3CR1+ CD8alpha+ dendritic cells are a steady-state population related to plasmacytoid dendritic cells. *Proc Natl Acad Sci U S A* (2010) **107**(33):14745–50. doi:10.1073/pnas.1001562107
49. Vu Manh T-P, Dalod M. Characterization of dendritic cell subsets through gene expression analysis. *Methods Mol Biol* (2015) (in press).
50. Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, et al. Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. *Immunity* (2012) **37**(1):60–73. doi:10.1016/j.immuni.2012.04.012
51. Chu CC, Ali N, Karagiannis P, Di Meglio P, Skowera A, Napolitano L, et al. Resident CD141 (BDCA3)+ dendritic cells in human skin produce IL-10 and induce regulatory T cells that suppress skin inflammation. *J Exp Med* (2012) **209**(5):935–45. doi:10.1084/jem.20112583
52. Dutertre CA, Jourdain JP, Rancez M, Amraoui S, Fossum E, Bogen B, et al. TLR3-responsive, XCR1+, CD141(BDCA-3)+/CD8alpha+-equivalent dendritic cells uncovered in healthy and simian immunodeficiency virus-infected rhesus macaques. *J Immunol* (2014) **192**(10):4697–708. doi:10.4049/jimmunol.1302448
53. Dutertre CA, Wang LF, Ginhoux F. Aligning bona fide dendritic cell populations across species. *Cell Immunol* (2014) **291**(1–2):3–10. doi:10.1016/j.cellimm.2014.08.006
54. Mildner A, Jung S. Development and function of dendritic cell subsets. *Immunity* (2014) **40**(5):642–56. doi:10.1016/j.immuni.2014.04.016
55. Guilliams M, Henri S, Tamoutounour S, Ardouin L, Schwartz-Cornil I, Dalod M, et al. From skin dendritic cells to a simplified classification of human and mouse dendritic cell subsets. *Eur J Immunol* (2010) **40**(8):2089–94. doi:10.1002/eji.201040498
56. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* (2010) **116**(16):e74–80. doi:10.1182/blood-2010-02-258558
57. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* (2014) **14**(8):571–8. doi:10.1038/nri3712
58. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* (2013) **31**:563–604. doi:10.1146/annurev-immunol-020711-074950
59. Bachem A, Hartung E, Guttler S, Mora A, Zhou X, Hegemann A, et al. Expression of XCR1 characterizes the Batf3-dependent lineage of dendritic cells capable of antigen cross-presentation. *Front Immunol* (2012) **3**:214. doi:10.3389/fimmu.2012.00214
60. Becker M, Guttler S, Bachem A, Hartung E, Mora A, Jakel A, et al. Ontogenic, phenotypic, and functional characterization of XCR1(+) dendritic cells leads to a consistent classification of intestinal dendritic cells based on the expression of XCR1 and SIRPalpha. *Front Immunol* (2014) **5**:326. doi:10.3389/fimmu.2014.00326
61. Tamoutounour S, Henri S, Lelouard H, de Bovis B, de Haar C, van der Woude CJ, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol* (2012) **42**(12):3150–66. doi:10.1002/eji.201242847
62. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* (2013) **38**(5):970–83. doi:10.1016/j.immuni.2013.04.011
63. Persson EK, Uronen-Hansson H, Semmrich M, Rivollier A, Hagerbrand K, Marsal J, et al. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* (2013) **38**(5):958–69. doi:10.1016/j.immuni.2013.03.009
64. Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* (2013) **154**(4):843–58. doi:10.1016/j.cell.2013.07.014
65. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen JL, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**(6):1261–71. doi:10.1084/jem.20092618
66. Lee J, Breton G, Oliveira TY, Zhou YJ, Aljoufi A, Puhr S, et al. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. *J Exp Med* (2015) **212**(3):385–99. doi:10.1084/jem.20141442
67. Schotte R, Nagasawa M, Weijer K, Spits H, Blom B. The ETS transcription factor Spi-B is required for human plasmacytoid dendritic cell development. *J Exp Med* (2004) **200**(11):1503–9. doi:10.1084/jem.20041231
68. Poulin LF, Reyal Y, Uronen-Hansson H, Schraml BU, Sancho D, Murphy KM, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. *Blood* (2012) **119**(25):6052–62. doi:10.1182/blood-2012-01-406967
69. Breton G, Lee J, Zhou YJ, Schreiber JJ, Keler T, Puhr S, et al. Circulating precursors of human CD1c+ and CD141+ dendritic cells. *J Exp Med* (2015) **212**(3):401–13. doi:10.1084/jem.20141441
70. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. IRF8 mutations and human dendritic-cell immunodeficiency. *N Engl J Med* (2011) **365**(2):127–38. doi:10.1056/NEJMoa1100066
71. Jardine L, Barge D, Ames-Draycott A, Pagan S, Cookson S, Spickett G, et al. Rapid detection of dendritic cell and monocyte disorders using CD4 as a lineage marker of the human peripheral blood antigen-presenting cell compartment. *Front Immunol* (2013) **4**:495. doi:10.3389/fimmu.2013.00495
72. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell* (2014) **26**(5):638–52. doi:10.1016/j.ccell.2014.09.007
73. Woo SR, Corrales L, Gajewski TF. Innate immune recognition of cancer. *Annu Rev Immunol* (2015) **33**:445–74. doi:10.1146/annurev-immunol-032414-112043
74. Summerfield A, Auray G, Ricklin M. Comparative dendritic cell biology of veterinary mammals. *Annu Rev Anim Biosci* (2015) **3**:533–57. doi:10.1146/annurev-animal-022114-111009
75. Vremec D, Hansen J, Strasser A, Acha-Orbea H, Zhan Y, O'Keeffe M, et al. Maintaining dendritic cell viability in culture. *Mol Immunol* (2015) **63**(2):264–7. doi:10.1016/j.molimm.2014.07.011
76. Contreras V, Urien C, Guiton R, Alexandre Y, Vu Manh TP, Andrieu T, et al. Existence of CD8alpha-like dendritic cells with a conserved functional

specialization and a common molecular signature in distant mammalian species. *J Immunol* (2010) **185**(6):3313–25. doi:10.4049/jimmunol.1000824
77. Fanning SL, George TC, Feng D, Feldman SB, Megjugorac NJ, Izaguirre AG, et al. Receptor cross-linking on human plasmacytoid dendritic cells leads to the regulation of IFN-alpha production. *J Immunol* (2006) **177**(9):5829–39. doi:10.4049/jimmunol.177.9.5829
78. Jahn PS, Zanker KS, Schmitz J, Dzionek A. BDCA-2 signaling inhibits TLR-9-agonist-induced plasmacytoid dendritic cell activation and antigen presentation. *Cell Immunol* (2010) **265**(1):15–22. doi:10.1016/j.cellimm.2010.06.005
79. Tavano B, Boasso A. Effect of immunoglobin-like transcript 7 cross-linking on plasmacytoid dendritic cells differentiation into antigen-presenting cells. *PLoS One* (2014) **9**(2):e89414. doi:10.1371/journal.pone.0089414
80. Duffy D, Rouilly V, Libri V, Hasan M, Beitz B, David M, et al. Functional analysis via standardized whole-blood stimulation systems defines the boundaries of a healthy immune response to complex stimuli. *Immunity* (2014) **40**(3):436–50. doi:10.1016/j.immuni.2014.03.002
81. Achim K, Arendt D. Structural evolution of cell types by step-wise assembly of cellular modules. *Curr Opin Genet Dev* (2014) **27**:102–8. doi:10.1016/j.gde.2014.05.001
82. Arendt D. The evolution of cell types in animals: emerging principles from molecular studies. *Nat Rev Genet* (2008) **9**(11):868–82. doi:10.1038/nrg2416
83. Vu Manh TP, Marty H, Sibille P, Le Vern Y, Kaspers B, Dalod M, et al. Existence of conventional dendritic cells in *Gallus gallus* revealed by comparative gene expression profiling. *J Immunol* (2014) **192**(10):4510–7. doi:10.4049/jimmunol.1303405
84. Marquet F, Vu Manh TP, Maisonnasse P, Elhmouzi-Younes J, Urien C, Bouguyon E, et al. Pig skin includes dendritic cell subsets transcriptomically related to human CD1a and CD14 dendritic cells presenting different migrating behaviors and T cell activation capacities. *J Immunol* (2014) **193**(12):5883–93. doi:10.4049/jimmunol.1303150
85. Crozat K, Guiton R, Guilliams M, Henri S, Baranek T, Schwartz-Cornil I, et al. Comparative genomics as a tool to reveal functional equivalences between human and mouse dendritic cell subsets. *Immunol Rev* (2010) **234**(1):177–98. doi:10.1111/j.0105-2896.2009.00868.x
86. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* (2010) **207**(6):1247–60. doi:10.1084/jem.20092140
87. Dudziak D, Kamphorst AO, Heidkamp GF, Buchholz VR, Trumpfheller C, Yamazaki S, et al. Differential antigen processing by dendritic cell subsets in vivo. *Science* (2007) **315**(5808):107–11. doi:10.1126/science.1136080
88. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* (2010) **33**(3):375–86. doi:10.1016/j.immuni.2010.08.012
89. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. *Blood* (2010) **115**(3):e10–9. doi:10.1182/blood-2009-07-235028
90. Segura E, Touzot M, Bohineust A, Cappuccio A, Chiocchia G, Hosmalin A, et al. Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity* (2013) **38**(2):336–48. doi:10.1016/j.immuni.2012.10.018
91. Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science* (2014) **343**(6172):776–9. doi:10.1126/science.1247651
92. Usoskin D, Furlan A, Islam S, Abdo H, Lonnerberg P, Lou D, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* (2015) **18**(1):145–53. doi:10.1038/nn.3881
93. Zeisel A, Manchado ABM, Codeluppi S, Lönnerberg P, La Manno G, Juréus A, et al. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science* (2015) **347**(6226):1138–42. doi:10.1126/science.aaa1934
94. Schakel K, Kannagi R, Kniep B, Goto Y, Mitsuoka C, Zwirner J, et al. 6-Sulfo LacNAc, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human dendritic cells. *Immunity* (2002) **17**(3):289–301. doi:10.1016/S1074-7613(02)00393-X
95. Lindstedt M, Lundberg K, Borrebaeck CA. Gene family clustering identifies functionally associated subsets of human in vivo blood and tonsillar dendritic cells. *J Immunol* (2005) **175**(8):4839–46. doi:10.4049/jimmunol.175.8.4839
96. Dobel T, Kunze A, Babatz J, Trankner K, Ludwig A, Schmitz M, et al. FcgammaRIII (CD16) equips immature 6-sulfo LacNAc-expressing dendritic cells (slanDCs) with a unique capacity to handle IgG-complexed antigens. *Blood* (2013) **121**(18):3609–18. doi:10.1182/blood-2012-08-447045
97. Vu Manh TP, Alexandre Y, Baranek T, Crozat K, Dalod M. Plasmacytoid, conventional, and monocyte-derived dendritic cells undergo a profound and convergent genetic reprogramming during their maturation. *Eur J Immunol* (2013) **43**(7):1706–15. doi:10.1002/eji.201243106
98. Reizis B, Bunin A, Ghosh HS, Lewis KL, Sisirak V. Plasmacytoid dendritic cells: recent progress and open questions. *Annu Rev Immunol* (2011) **29**:163–83. doi:10.1146/annurev-immunol-031210-101345
99. Watchmaker PB, Lahl K, Lee M, Baumjohann D, Morton J, Kim SJ, et al. Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. *Nat Immunol* (2014) **15**(1):98–108. doi:10.1038/ni.2768
100. Lauterbach H, Bathke B, Gilles S, Traidl-Hoffmann C, Luber CA, Fejer G, et al. Mouse CD8alpha+ DCs and human BDCA3+ DCs are major producers of IFN-lambda in response to poly IC. *J Exp Med* (2010) **207**(12):2703–17. doi:10.1084/jem.20092720
101. Lundberg K, Albrekt AS, Nelissen I, Santegoets S, de Gruijl TD, Gibbs S, et al. Transcriptional profiling of human dendritic cell populations and models – unique profiles of in vitro dendritic cells and implications on functionality and applicability. *PLoS One* (2013) **8**(1):e52875. doi:10.1371/journal.pone.0052875
102. Reizis B. Regulation of plasmacytoid dendritic cell development. *Curr Opin Immunol* (2010) **22**(2):206–11. doi:10.1016/j.coi.2010.01.005
103. Villadangos JA, Shortman K. Found in translation: the human equivalent of mouse CD8+ dendritic cells. *J Exp Med* (2010) **207**(6):1131–4. doi:10.1084/jem.20100985
104. Naik SH. Demystifying the development of dendritic cell subtypes, a little. *Immunol Cell Biol* (2008) **86**(5):439–52. doi:10.1038/icb.2008.28
105. Soumelis V, Pattarini L, Michea P, Cappuccio A. Systems approaches to unravel innate immune cell diversity, environmental plasticity and functional specialization. *Curr Opin Immunol* (2015) **32C**:42–7. doi:10.1016/j.coi.2014.12.007
106. Deauvieau F, Ollion V, Doffin AC, Achard C, Fonteneau JF, Verronese E, et al. Human natural killer cells promote cross-presentation of tumor cell-derived antigens by dendritic cells. *Int J Cancer* (2015) **136**(5):1085–94. doi:10.1002/ijc.29087
107. Flinsenberg TW, Compeer EB, Koning D, Klein M, Amelung FJ, van Baarle D, et al. Fcgamma receptor antigen targeting potentiates cross-presentation by human blood and lymphoid tissue BDCA-3+ dendritic cells. *Blood* (2012) **120**(26):5163–72. doi:10.1182/blood-2012-06-434498
108. Nizzoli G, Krietsch J, Weick A, Steinfelder S, Facciotti F, Gruarin P, et al. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* (2013) **122**(6):932–42. doi:10.1182/blood-2013-04-495424
109. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, Manz MG. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol* (2007) **8**(11):1207–16. doi:10.1038/ni1518
110. Naik SH, Sathe P, Park HY, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol* (2007) **8**(11):1217–26. doi:10.1038/ni1522
111. Thordardottir S, Hangalapura BN, Hutten T, Cossu M, Spanholtz J, Schaap N, et al. The aryl hydrocarbon receptor antagonist StemRegenin 1 promotes human plasmacytoid and myeloid dendritic cell development from CD34+ hematopoietic progenitor cells. *Stem Cells Dev* (2014) **23**(9):955–67. doi:10.1089/scd.2013.0521
112. Proietto AI, Mittag D, Roberts AW, Sprigg N, Wu L. The equivalents of human blood and spleen dendritic cell subtypes can be generated in vitro from human CD34(+) stem cells in the presence of fms-like tyrosine kinase 3 ligand and thrombopoietin. *Cell Mol Immunol* (2012) **9**(6):446–54. doi:10.1038/cmi.2012.48

113. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* (2008) **135**(1):37–48. doi:10.1016/j.cell.2008.09.016
114. Sawai CM, Sisirak V, Ghosh HS, Hou EZ, Ceribelli M, Staudt LM, et al. Transcription factor Runx2 controls the development and migration of plasmacytoid dendritic cells. *J Exp Med* (2013) **210**(11):2151–9. doi:10.1084/jem.20130443
115. Esashi E, Bao M, Wang YH, Cao W, Liu YJ. PACSIN1 regulates the TLR7/9-mediated type I interferon response in plasmacytoid dendritic cells. *Eur J Immunol* (2012) **42**(3):573–9. doi:10.1002/eji.201142045
116. Ippolito GC, Dekker JD, Wang YH, Lee BK, Shaffer AL III, Lin J, et al. Dendritic cell fate is determined by BCL11A. *Proc Natl Acad Sci U S A* (2014) **111**(11):E998–1006. doi:10.1073/pnas.1319228111
117. Wu X, Satpathy AT, Kc W, Liu P, Murphy TL, Murphy KM. Bcl11a controls Flt3 expression in early hematopoietic progenitors and is required for pDC development in vivo. *PLoS One* (2013) **8**(5):e64800. doi:10.1371/journal.pone.0064800
118. Meredith MM, Liu K, Darrasse-Jeze G, Kamphorst AO, Schreiber HA, Guermonprez P, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J Exp Med* (2012) **209**(6):1153–65. doi:10.1084/jem.20112675
119. Satpathy AT, Kc W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med* (2012) **209**(6):1135–52. doi:10.1084/jem.20120030
120. Ohtsuka H, Sakamoto A, Pan J, Inage S, Horigome S, Ichii H, et al. Bcl6 is required for the development of mouse CD4+ and CD8alpha+ dendritic cells. *J Immunol* (2011) **186**(1):255–63. doi:10.4049/jimmunol.0903714
121. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* (2008) **322**(5904):1097–100. doi:10.1126/science.1164206
122. Nair-Gupta P, Baccarini A, Tung N, Seyffer F, Florey O, Huang Y, et al. TLR signals induce phagosomal MHC-I delivery from the endosomal recycling compartment to allow cross-presentation. *Cell* (2014) **158**(3):506–21. doi:10.1016/j.cell.2014.04.054
123. Avram D, Califano D. The multifaceted roles of Bcl11b in thymic and peripheral T cells: impact on immune diseases. *J Immunol* (2014) **193**(5):2059–65. doi:10.4049/jimmunol.1400930
124. Fu G, Vallee S, Rybakin V, McGuire MV, Ampudia J, Brockmeyer C, et al. Themis controls thymocyte selection through regulation of T cell antigen receptor-mediated signaling. *Nat Immunol* (2009) **10**(8):848–56. doi:10.1038/ni.1766
125. Johnson AL, Aravind L, Shulzhenko N, Morgun A, Choi SY, Crockford TL, et al. Themis is a member of a new metazoan gene family and is required for the completion of thymocyte positive selection. *Nat Immunol* (2009) **10**(8):831–9. doi:10.1038/ni.1769
126. Kakugawa K, Yasuda T, Miura I, Kobayashi A, Fukiage H, Satoh R, et al. A novel gene essential for the development of single positive thymocytes. *Mol Cell Biol* (2009) **29**(18):5128–35. doi:10.1128/MCB.00793-09
127. Lesourne R, Uehara S, Lee J, Song KD, Li L, Pinkhasov J, et al. Themis, a T cell-specific protein important for late thymocyte development. *Nat Immunol* (2009) **10**(8):840–7. doi:10.1038/ni.1768
128. Meredith MM, Liu K, Kamphorst AO, Idoyaga J, Yamane A, Guermonprez P, et al. Zinc finger transcription factor zDC is a negative regulator required to prevent activation of classical dendritic cells in the steady state. *J Exp Med* (2012) **209**(9):1583–93. doi:10.1084/jem.20121003
129. Yoshio S, Kanto T, Kuroda S, Matsubara T, Higashitani K, Kakita N, et al. Human blood dendritic cell antigen 3 (BDCA3)(+) dendritic cells are a potent producer of interferon-lambda in response to hepatitis C virus. *Hepatology* (2013) **57**(5):1705–15. doi:10.1002/hep.26182
130. Zhang S, Kodys K, Li K, Szabo G. Human type 2 myeloid dendritic cells produce interferon-lambda and amplify interferon-alpha in response to hepatitis C virus infection. *Gastroenterology* (2013) **144**(2):414–25e7. doi:10.1053/j.gastro.2012.10.034
131. Cao W, Bover L, Cho M, Wen X, Hanabuchi S, Bao M, et al. Regulation of TLR7/9 responses in plasmacytoid dendritic cells by BST2 and ILT7 receptor interaction. *J Exp Med* (2009) **206**(7):1603–14. doi:10.1084/jem.20090547
132. Kolodkin AL, Levengood DV, Rowe EG, Tai YT, Giger RJ, Ginty DD. Neuropilin is a semaphorin III receptor. *Cell* (1997) **90**(4):753–62. doi:10.1016/S0092-8674(00)80535-8
133. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* (1998) **92**(6):735–45. doi:10.1016/S0092-8674(00)81402-6
134. Nascimbeni M, Perie L, Chorro L, Diocou S, Kreitmann L, Louis S, et al. Plasmacytoid dendritic cells accumulate in spleens from chronically HIV-infected patients but barely participate in interferon-alpha expression. *Blood* (2009) **113**(24):6112–9. doi:10.1182/blood-2008-07-170803
135. Baranek T, Manh TP, Alexandre Y, Maqbool MA, Cabeza JZ, Tomasello E, et al. Differential responses of immune cells to type I interferon contribute to host resistance to viral infection. *Cell Host Microbe* (2012) **12**(4):571–84. doi:10.1016/j.chom.2012.09.002
136. Ruscanu S, Jouneau L, Urien C, Bourge M, Lecardonnel J, Moroldo M, et al. Dendritic cell subtypes from lymph nodes and blood show contrasted gene expression programs upon Bluetongue virus infection. *J Virol* (2013) **87**(16):9333–43. doi:10.1128/JVI.00631-13
137. Banchereau R, Baldwin N, Cepika AM, Athale S, Xue Y, Yu CI, et al. Transcriptional specialization of human dendritic cell subsets in response to microbial vaccines. *Nat Commun* (2014) **5**:5283. doi:10.1038/ncomms6283
138. Shalek AK, Satija R, Adiconis X, Gertner RS, Gaublomme JT, Raychowdhury R, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. *Nature* (2013) **498**(7453):236–40. doi:10.1038/nature12172
139. Venkatesh B, Lee AP, Ravi V, Maurya AK, Lian MM, Swann JB, et al. Elephant shark genome provides unique insights into gnathostome evolution. *Nature* (2014) **505**(7482):174–9. doi:10.1038/nature12826
140. van der Aa E, van Montfoort N, Woltman AM. BDCA3CLEC9A human dendritic cell function and development. *Semin Cell Dev Biol* (2014) (in press). doi:10.1016/j.semcdb.2014.05.016
141. Gallois A, Bhardwaj N. A needle in the 'cancer vaccine' haystack. *Nat Med* (2010) **16**(8):854–6. doi:10.1038/nm0810-854
142. Tullett KM, Lahoud MH, Radford KJ. Harnessing human cross-presenting CLEC9A(+)XCR1(+) dendritic cells for immunotherapy. *Front Immunol* (2014) **5**:239. doi:10.3389/fimmu.2014.00239
143. Meixlsperger S, Leung CS, Ramer PC, Pack M, Vanoaica LD, Breton G, et al. CD141+ dendritic cells produce prominent amounts of IFN-alpha after dsRNA recognition and can be targeted via DEC-205 in humanized mice. *Blood* (2013) **121**(25):5034–44. doi:10.1182/blood-2012-12-473413
144. Sancho D, Joffre OP, Keller AM, Rogers NC, Martinez D, Hernanz-Falcon P, et al. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. *Nature* (2009) **458**(7240):899–903. doi:10.1038/nature07750
145. Zelenay S, Keller AM, Whitney PG, Schraml BU, Deddouche S, Rogers NC, et al. The dendritic cell receptor DNGR-1 controls endocytic handling of necrotic cell antigens to favor cross-priming of CTLs in virus-infected mice. *J Clin Invest* (2012) **122**(5):1615–27. doi:10.1172/JCI60644
146. Huysamen C, Willment JA, Dennehy KM, Brown GD. CLEC9A is a novel activation C-type lectin-like receptor expressed on BDCA3+ dendritic cells and a subset of monocytes. *J Biol Chem* (2008) **283**(24):16693–701. doi:10.1074/jbc.M709923200
147. Tel J, Aarntzen EH, Baba T, Schreibelt G, Schulte BM, Benitez-Ribas D, et al. Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. *Cancer Res* (2013) **73**(3):1063–75. doi:10.1158/0008-5472.CAN-12-2583
148. Tel J, Sittig SP, Blom RA, Cruz LJ, Schreibelt G, Figdor CG, et al. Targeting uptake receptors on human plasmacytoid dendritic cells triggers antigen cross-presentation and robust type I IFN secretion. *J Immunol* (2013) **191**(10):5005–12. doi:10.4049/jimmunol.1300787
149. Guery L, Hugues S. Tolerogenic and activatory plasmacytoid dendritic cells in autoimmunity. *Front Immunol* (2013) **4**:59. doi:10.3389/fimmu.2013.00059
150. Goubier A, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, et al. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* (2008) **29**(3):464–75. doi:10.1016/j.immuni.2008.06.017
151. Yu CI, Becker C, Wang Y, Marches F, Helft J, Leboeuf M, et al. Human CD1c+ dendritic cells drive the differentiation of CD103+ CD8+ mucosal

effector T cells via the cytokine TGF-beta. *Immunity* (2013) **38**(4):818–30. doi:10.1016/j.immuni.2013.03.004

152. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. *J Exp Med* (2011) **208**(10):2005–16. doi:10.1084/jem.20101159
153. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, et al. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. *J Exp Med* (2011) **208**(10):1989–2003. doi:10.1084/jem.20101158

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Blood monocytes and their subsets: established features and open questions

*Loems Ziegler-Heitbrock**

Helmholtz Zentrum München, Asklepios Fachkliniken München-Gauting, Gauting, Germany

In contrast to the past reliance on morphology, the identification and enumeration of blood monocytes are nowadays done with monoclonal antibodies and flow cytometry and this allows for subdivision into classical, intermediate, and non-classical monocytes. Using specific cell surface markers, dendritic cells in blood can be segregated from these monocytes. While in the past, changes in monocyte numbers as determined in standard hematology counters have not had any relevant clinical impact, the subset analysis now has uncovered informative changes that may be used in management of disease.

Keywords: monocyte subsets, nomenclature, classical monocytes, intermediate monocytes, non-classical monocytes

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Anne Hosmalin, Cochin Institute, France
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Claudia Jakubzick, National Jewish Health, USA

***Correspondence:**
Loems Ziegler-Heitbrock, Helmholtz Zentrum München, Asklepios Fachkliniken München-Gauting, Enzianstr.3, 82211 Herrsching, Germany
lzh@monocyte.eu

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The Definition of Monocytes

The term monocyte is used for blood cells of a lineage called monocytes/macrophages or mononuclear phagocytes. These blood monocytes are bone marrow-derived leukocytes that are functionally characterized by the ability to phagocytose, to produce cytokines, and to present antigen. In early studies, they had been identified based on glass adherence and morphology (1). Also, cytochemistry for specific enzymes like monocyte-specific esterase (2, 3) has been employed, while the standard approach in clinical hematology relies on physical properties of these cells including light scatter.

In bone marrow, the monocytes derive from myelo-monocytic stem cells, which give rise to more direct precursors like monoblasts and pro-monocytes. These cells earlier were identified based on morphology (4) such that the monoblast was an ill-defined cell type. More recently in the mouse model, a Ly6C+ CD115+ CD117+ monoblast-type cell, termed common monocyte progenitor (cMoP), was identified in bone marrow and spleen and this cell is able to proliferate and give rise to the different monocyte subsets (5). A cMoP monoblast type of cell remains to be identified for man and other species.

The number of circulating blood monocytes in man can strongly increase within minutes by stress or exercise followed by a rapid return to baseline levels. These recruited cells are thought to come from what is called the marginal pool (6). This compartment describes areas of reduced blood velocity close to the endothelium of venules and here cells can loosely adhere and can be mobilized in a catecholamine-dependent fashion (7). These marginal pool monocytes can have an adhesion molecule pattern distinct from monocytes found in blood at rest.

In addition, CD11b^{high} (CD90, B220, CD49b, NK1.1, Ly-6G, F4/80, I-Ab, CD11c)^{low} cells are mobilized from the spleen after severe injury (8). These cells have monocyte morphology and their transcriptome matches with that of blood monocytes. Furthermore, CD11b+ Ly6C^{hi} monocytes can be mobilized from bone marrow to blood in infectious disease models (9), and adoptively transferred monocytes were shown to return to the bone marrow (10) in the mouse. What remains to be determined is whether the spleen and bone marrow compartments also contribute to the pool of monocytes that can be mobilized by stress and exercise.

When under homeostatic or inflammatory conditions, the monocytes have migrated into tissue; then by definition, these cells are called macrophages. Cells newly emigrated into the lung have been termed monocytes in some studies [e.g., Ref. (11)]. Since monocytes, once they have arrived in tissue, will start to transform into larger cells and rapidly lose their monocyte characteristics, others have called these recently emigrated cells "small macrophages" (12).

More detailed studies in the mouse have demonstrated tissue cells with characteristics close to blood monocytes (13, 14). However, these cells in the lymph node show a gene expression pattern that distinguishes them from the blood cells (14) and in the skin they show increased expression of lysozyme and CD68, markers typical of mature macrophages (13). Therefore, more data are required in the mouse model and obviously also in man before a consensus can be reached whether we use the term tissue monocyte or whether we continue to call these cells macrophages. Until these issues have been resolved, the term monocyte should be restricted to cells in the blood compartment and the bone marrow and spleen reservoirs that can replenish the blood monocyte pool.

Definition of Blood Monocytes Based on Cells Surface Markers

As explained above, monocytes initially had been identified by function and morphology and these criteria have been misleading especially when disease processes altered these features. Therefore, attempts have been made to define unequivocal criteria for monocytes. Here, monoclonal antibodies against cell surface molecules have been proposed. In man, CD14 has been used as a marker (15), and in the mouse, CD115 is often employed (16). CD115 identifies the M-CSF receptor and has the main drawback that in the mouse, it is downregulated on blood monocytes with inflammation (17). Also, the question is whether such markers are sufficiently specific and do not react with other cell types like dendritic cells (DCs). In fact, part of the CD1c+ blood DCs in man can express low-level CD14 (18) and also human B cells have been reported to express some CD14 (19). Therefore, monocytes can be identified with markers like CD14 and CD115, but this should be supported by additional markers and by functional studies. Interestingly, when searching for macrophage-specific transcripts in the mouse, CD64 and MerTK have emerged (20). While CD64 is absent from non-classical monocytes in man, MerTK is a molecule that might prove informative for blood monocytes in different species. In addition, staining for CD16, which is used for monocyte subset definition (see below), will at the same time help to exclude DCs in human blood.

Dissection of Monocytes from Dendritic Cells

Dendritic cells were first described by Steinman and Cohn as stellate cells isolated from mouse spleen (21). Over the years, there have been debates as to whether these cells are a distinct lineage or part of the mononuclear phagocyte system. A common precursor for monocytes and DCs was described in the mouse (22), but the existence of this cell was later disputed (23) suggesting that DCs and monocytes may diverge at an earlier multi-potent progenitor stage (24).

However, the demonstration that monocytes can be used to generate DCs *in vitro* by adding GM-CSF and IL-4 suggested a close relationship between monocytes and DCs (25). Later, transcriptome analysis demonstrated that such monocyte-derived DCs rather resemble macrophages than DCs from lymphoid tissue (26). Therefore, these *in vitro* generated monocyte-derived cells are potent antigen-presenting cells, but they do not represent *bona fide* DCs; they rather belong to the monocyte/macrophage lineage. Still not resolved is the question whether in tissue the monocyte-derived cells with high levels of class II expression and with high antigen-presenting capacity should be termed monocyte-derived DC (13, 27, 28) or activated macrophages.

In addition to DCs in tissue, cells with DC properties have been described in blood based on the expression of CD68, CD1c, or CD141 (29, 30). Transcriptome analysis has demonstrated that these cells and the monocytes belong to different clusters (26, 31). These data suggest that blood DCs can be segregated from monocytes and macrophages as a separate lineage.

The data also demonstrate the power of transcriptomic analysis in defining and dissecting leukocyte populations like monocytes and DCs. Ontogeny can help in such a definition, but in men, adoptive transfer is limited to strategies like transfer of bone marrow stem cells, and informative mutations are rare. Also, the ontogeny approach needs to be used with caution since a defined progenitor cell can give rise to clearly distinct cell populations. An informative example is the megakaryocyte–erythrocyte progenitor (MEP) cell, which gives rise to either megakaryocytes and their platelet progeny or to erythroblasts and their red blood cell progeny (32, 33). Megakaryocytes and erythroblasts have a distinct transcriptome (34), and they are involved in distinct functions, i.e., in blood clotting and oxygen transport, respectively. Therefore, although having a common ontogeny, these cells belong to clearly separate lineages. This example illustrates that ontogeny can provide a framework, but a comprehensive analyses like transcriptomics and the analysis of cell function are required for dissecting cell types and for developing a nomenclature. Therefore, in order to assign a novel leukocyte population in blood or tissue to either monocytes or DCs, a straight-forward approach is to analyze the transcriptome (and other omics like the proteome, lipidome, glycome, or metabolome) of these cells in comparison to typical monocytes and DCs and to then ask whether the novel cell type co-clusters with either prototypic monocytes or DCs (26).

Monocyte Subpopulations

Evidence for monocyte subpopulations has come from experiments using differential flotation in counter-current elutriation (35) and from differential binding to antibody-coated red blood cells, which has defined populations with different functions (36). With the use of monoclonal antibodies and flow cytometry, tools have become available to clearly define, enumerate, and isolate monocyte subsets based on the differential expression of CD14 and CD16 cell-surface markers (37).

In 2010, an international consortium under the auspices of the IUIS and the WHO has proposed a nomenclature for monocyte

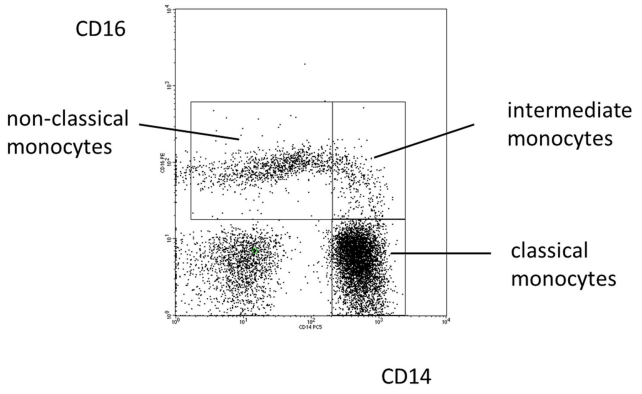

FIGURE 1 | Blood monocyte subsets in man. Illustration of the definition of human monocyte subsets in health based on a typical distribution of events in a CD14 CD16 staining.

subpopulations (38). The proposal defined the major population of $CD14^{high}$ cells found in human blood as classical monocytes and the minor population of cells with low CD14 and high CD16 as non-classical monocytes. A population in between these two subsets was termed intermediate monocytes (see **Figure 1**).

While an unequivocal approach to defining the intermediate monocytes has not been developed, as yet (39), a host of studies on intermediate monocytes has been published since the 2010 proposal. In fact, a search for the term "intermediate monocyte" under Google Scholar has revealed more than 100 studies on these cells since 2010. These reports have described an expansion of intermediate monocytes in various inflammatory diseases and these cells have been shown to be of prognostic relevance in cardiovascular disease (40). The use of additional markers for delineation of intermediate monocytes has been suggested (41) and it remains to be shown whether markers, such as CCR2 or slan, will improve the definition of these cells.

The same nomenclature as proposed for man can be used in other species [reviewed in Ref. (42)]. The respective cells can be very similar to men as seen for non-human primates (43, 44). In species like the mouse, the classical and non-classical monocyte subsets can be identified as well, but different markers like CD115, Ly6C, and CD43 are used (16, 45). Also in species like rat, pig, cow, and horse, classical and non-classical monocytes can be defined and even intermediate monocytes have been described in some animals (42). It is predicted that the nomenclature of monocyte subsets will be applicable to all mammalian species.

In human blood, a population of slan-positive cells has been described as DCs, but phenotypic analysis has shown that these cells are CD14-low and CD16-high (46), functional studies demonstrated a high capacity to produce TNF (47), and clinical studies showed that these cells are depleted by glucocorticoid treatment (48). These features are identical to what has been reported as characteristics of non-classical CD14+CD16++ monocytes (37, 49, 50). Also, the increased absolute numbers of slan-positive monocytes and of non-classical monocytes show a clear correlation in HIV-infected patients (51), and part of the non-classical monocytes has been shown to be slan-positive (52–54). Collectively, these findings suggest that the slan-positive cells belong to the non-classical monocytes.

There may be additional monocyte subsets including Fcepsilon-RI-positive cells (55), which were found with a median of 2.5% among CD14-positive blood monocytes in a pediatric cohort (56) and these cells may be involved in IgE clearance (57). Also, proliferating monocytes have been described (58) as well as precursors for fibrocytes (59) and osteoclasts (60). For all of these cell types, further characterization is awaited.

Clinical Implications of Monocyte Numbers

Monocyte numbers as defined in the hematology lab using light scatter properties have not contributed much to diagnosis and monitoring of disease, but with the definition of monocyte subsets by flow cytometry, informative patterns have emerged. For example, severe infection will increase the number of non-classical and intermediate monocytes (61–63). Here, it remains to be analyzed whether such an increase can predict prognosis, as has been suggested (64). Furthermore, therapy with glucocorticoids leads to a decrease of non-classical monocytes, which appears to be due to a selective induction of apoptosis in the non-classical monocytes while classical monocytes even increase in number under glucocorticoids (50, 65). Also, blockade of the M-CSF pathway can lead to depletion of non-classical monocytes (66–68). A likely explanation is that M-CSF signaling via the CD115 M-CSF receptor is required for the classical monocytes to mature into non-classical monocytes. Again still to be determined is whether such a drug-induced depletion can be used to predict therapeutic response in inflammatory diseases. Still unresolved is the mechanism of depletion of non-classical monocytes in three siblings within one family (69). Here, more families with this type of defect need to be analyzed in order to identify the gene and the mechanisms involved. Finally, the absolute count of intermediate monocytes was shown to predict cardiovascular events (70, 71). Hence, analysis of monocyte subsets by flow cytometry now provides clinically useful parameters in various settings. What remains to be established in this context is an unequivocal dissection of the non-classical and the intermediate monocytes.

References

1. van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. *J Exp Med* (1968) **128**:415–35. doi:10.1084/jem.128.3.415
2. Tucker SB, Pierre RV, Jordon RE. Rapid identification of monocytes in a mixed mononuclear cell preparation. *J Immunol Methods* (1977) **14**:267–9. doi:10.1016/0022-1759(77)90137-5
3. Uphoff CC, Drexler HG. Biology of monocyte-specific esterase. *Leuk Lymphoma* (2000) **39**:257–70. doi:10.3109/10428190009065825
4. Goud TJ, Schotte C, van Furth R. Identification and characterization of the monoblast in mononuclear phagocyte colonies grown in vitro. *J Exp Med* (1975) **142**:1180–99. doi:10.1084/jem.142.5.1180
5. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* (2013) **14**:821–30. doi:10.1038/ni.2638
6. Klonz A, Wonigeit K, Pabst R, Westermann J. The marginal blood pool of the rat contains not only granulocytes, but also lymphocytes, NK-cells and monocytes: a second intravascular compartment, its cellular composition, adhesion

molecule expression and interaction with the peripheral blood pool. *Scand J Immunol* (1996) **44**:461–9. doi:10.1046/j.1365-3083.1996.d01-334.x
7. Steppich B, Dayyani F, Gruber R, Lorenz R, Mack M, Ziegler-Heitbrock HWL. Selective mobilization of CD14(+)CD16(+) monocytes by exercise. *Am J Physiol Cell Physiol* (2000) **279**:C578–86.
8. Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science* (2009) **325**:612–6. doi:10.1126/science.1175202
9. Shi C, Jia T, Mendez-Ferrer S, Hohl TM, Serbina NV, Lipuma L, et al. Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. *Immunity* (2011) **34**:590–601. doi:10.1016/j.immuni.2011.02.016
10. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* (2007) **204**:171–80. doi:10.1084/jem.20061011
11. Alexis N, Soukup J, Ghio A, Becker S. Sputum phagocytes from healthy individuals are functional and activated: a flow cytometric comparison with cells in bronchoalveolar lavage and peripheral blood. *Clin Immunol* (2000) **97**:21–32. doi:10.1006/clim.2000.4911
12. Frankenberger M, Eder C, Hofer TP, Heimbeck I, Skokann K, Kassner G, et al. Chemokine expression by small sputum macrophages in COPD. *Mol Med* (2011) **17**:762–70. doi:10.2119/molmed.2010.00202
13. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* (2013) **39**:925–38. doi:10.1016/j.immuni.2013.10.004
14. Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, et al. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* (2013) **39**:599–610. doi:10.1016/j.immuni.2013.08.007
15. Ziegler-Heitbrock HWL, Ulevitch RJ. CD14: cell surface receptor and differentiation marker. *Immunol Today* (1993) **14**:121–5. doi:10.1016/0167-5699(93)90212-4
16. Sunderkotter C, Nikolic T, Dillon MJ, Van Rooijen N, Stehling M, Drevets DA, et al. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J Immunol* (2004) **172**:4410–7. doi:10.4049/jimmunol.172.7.4410
17. Drevets DA, Schawang JE, Mandava VK, Dillon MJ, Leenen PJ. Severe *Listeria* monocytogenes infection induces development of monocytes with distinct phenotypic and functional features. *J Immunol* (2010) **185**:2432–41. doi:10.4049/jimmunol.1000486
18. Schwarz H, Schmittner M, Duschl A, Horejs-Hoeck J. Residual endotoxin contaminations in recombinant proteins are sufficient to activate human CD1c+ dendritic cells. *PLoS One* (2014) **9**:e113840. doi:10.1371/journal.pone.0113840
19. Ziegler-Heitbrock HWL, Pechumer H, Petersmann I, Durieux JJ, Vita N, Labeta MO, et al. CD14 is expressed and functional in human B cells. *Eur J Immunol* (1994) **24**:1937–40. doi:10.1002/eji.1830240835
20. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* (2012) **13**:1118–28. doi:10.1038/ni.2419
21. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* (1973) **137**:1142–62. doi:10.1084/jem.137.5.1142
22. Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, Littman DR, et al. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* (2006) **311**:83–7. doi:10.1126/science.1117729
23. Sathe P, Metcalf D, Vremec D, Naik SH, Langdon WY, Huntington ND, et al. Lymphoid tissue and plasmacytoid dendritic cells and macrophages do not share a common macrophage-dendritic cell-restricted progenitor. *Immunity* (2014) **41**:104–15. doi:10.1016/j.immuni.2014.05.020
24. Onai N, Ohteki T. Bipotent or oligopotent? A macrophage and DC progenitor revisited. *Immunity* (2014) **41**:5–7. doi:10.1016/j.immuni.2014.07.004
25. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* (1994) **179**:1109–18. doi:10.1084/jem.179.4.1109
26. Robbins SH, Walzer T, Dembele D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* (2008) **9**:R17. doi:10.1186/gb-2008-9-1-r17
27. Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunity* (1999) **11**:753–61. doi:10.1016/S1074-7613(00)80149-1
28. Landsman L, Varol C, Jung S. Distinct differentiation potential of blood monocyte subsets in the lung. *J Immunol* (2007) **178**:2000–7. doi:10.4049/jimmunol.178.4.2000
29. Strobl H, Scheinecker C, Riedl E, Csmarits B, Bello-Fernandez C, Pickl WF, et al. Identification of CD68+lin- peripheral blood cells with dendritic precursor characteristics. *J Immunol* (1998) **161**:740–8.
30. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* (2000) **165**:6037–46. doi:10.4049/jimmunol.165.11.6037
31. Frankenberger M, Hofer TP, Marei A, Dayyani F, Schewe S, Strasser C, et al. Transcript profiling of CD16-positive monocytes reveals a unique molecular fingerprint. *Eur J Immunol* (2012) **42**:957–74. doi:10.1002/eji.201141907
32. Debili N, Coulombel L, Croisille L, Katz A, Guichard J, Breton-Gorius J, et al. Characterization of a bipotent erythro-megakaryocytic progenitor in human bone marrow. *Blood* (1996) **88**:1284–96.
33. Klimchenko O, Mori M, Distefano A, Langlois T, Larbret F, Lecluse Y, et al. A common bipotent progenitor generates the erythroid and megakaryocyte lineages in embryonic stem cell-derived primitive hematopoiesis. *Blood* (2009) **114**:1506–17. doi:10.1182/blood-2008-09-178863
34. Macaulay IC, Tijssen MR, Thijssen-Timmer DC, Gusnanto A, Steward M, Burns P, et al. Comparative gene expression profiling of in vitro differentiated megakaryocytes and erythroblasts identifies novel activatory and inhibitory platelet membrane proteins. *Blood* (2007) **109**:3260–9. doi:10.1182/blood-2006-07-036269
35. Norris DA, Morris RM, Sanderson RJ, Kohler PF. Isolation of functional subsets of human peripheral blood monocytes. *J Immunol* (1979) **123**:166–72.
36. Zembala M, Uracz W, Ruggiero I, Mytar B, Pryjma J. Isolation and functional characteristics of FcR+ and FcR- human monocyte subsets. *J Immunol* (1984) **133**:1293–9.
37. Passlick B, Flieger D, Ziegler-Heitbrock HWL. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* (1989) **74**:2527–34.
38. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* (2010) **116**:e74–80. doi:10.1182/blood-2010-02-258558
39. Ziegler-Heitbrock L, Hofer TP. Toward a refined definition of monocyte subsets. *Front Immunol* (2013) **4**:23. doi:10.3389/fimmu.2013.00023
40. Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, et al. CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography. *J Am Coll Cardiol* (2012) **60**:1512–20. doi:10.1016/j.jacc.2012.07.019
41. Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. *Immunol Res* (2012) **53**:41–57. doi:10.1007/s12026-012-8297-3
42. Ziegler-Heitbrock L. Monocyte subsets in man and other species. *Cell Immunol* (2014) **289**:135–9. doi:10.1016/j.cellimm.2014.03.019
43. Kim WK, Sun Y, Do H, Autissier P, Halpern EF, Piatak M Jr, et al. Monocyte heterogeneity underlying phenotypic changes in monocytes according to SIV disease stage. *J Leukoc Biol* (2010) **87**:557–67. doi:10.1189/jlb.0209082
44. Kwissa M, Nakaya HI, Oluoch H, Pulendran B. Distinct TLR adjuvants differentially stimulate systemic and local innate immune responses in nonhuman primates. *Blood* (2012) **119**:2044–55. doi:10.1182/blood-2011-10-388579
45. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. *Blood* (2010) **115**:e10–9. doi:10.1182/blood-2009-07-235028
46. de Baey A, Mende I, Riethmueller G, Baeuerle PA. Phenotype and function of human dendritic cells derived from M-DC8(+) monocytes. *Eur J Immunol* (2001) **31**:1646–55. doi:10.1002/1521-4141(200106)31:6<1646::AID-IMMU1646>3.0.CO;2-X

47. Schakel K, Kannagi R, Kniep B, Goto Y, Mitsuoka C, Zwirner J, et al. 6-Sulfo LacNAc, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human dendritic cells. *Immunity* (2002) **17**:289–301. doi:10.1016/S1074-7613(02)00393-X
48. Thomas K, Dietze K, Wehner R, Metz I, Tumani H, Schultheiss T, et al. Accumulation and therapeutic modulation of 6-sulfo LacNAc(+) dendritic cells in multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* (2014) **1**:e33. doi:10.1212/NXI.0000000000000033
49. Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, et al. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. *J Immunol* (2002) **168**:3536–42. doi:10.4049/jimmunol.168.7.3536
50. Fingerle-Rowson G, Angstwurm M, Andreesen R, Ziegler-Heitbrock HWL. Selective depletion of CD14+ CD16+ monocytes by glucocorticoid therapy. *Clin Exp Immunol* (1998) **112**:501–6. doi:10.1046/j.1365-2249.1998.00617.x
51. Dutertre CA, Amraoui S, DeRosa A, Jourdain JP, Vimeux L, Goguet M, et al. Pivotal role of M-DC8(+) monocytes from viremic HIV-infected patients in TNFalpha overproduction in response to microbial products. *Blood* (2012) **120**:2259–68. doi:10.1182/blood-2012-03-418681
52. Siedlar M, Frankenberger M, Ziegler-Heitbrock LH, Belge KU. The M-DC8-positive leukocytes are a subpopulation of the CD14+ CD16+ monocytes. *Immunobiology* (2000) **202**:11–7. doi:10.1016/S0171-2985(00)80047-9
53. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* (2010) **33**:375–86. doi:10.1016/j.immuni.2010.08.012
54. Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, et al. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood* (2011) **118**:e16–31. doi:10.1182/blood-2010-12-326355
55. Maurer D, Fiebiger E, Reininger B, Wolff-Winiski B, Jouvin MH, Kilgus O, et al. Expression of functional high affinity immunoglobulin E receptors (Fc epsilon RI) on monocytes of atopic individuals. *J Exp Med* (1994) **179**:745–50. doi:10.1084/jem.179.2.745
56. Dehlink E, Baker AH, Yen E, Nurko S, Fiebiger E. Relationships between levels of serum IgE, cell-bound IgE, and IgE-receptors on peripheral blood cells in a pediatric population. *PLoS One* (2010) **5**:e12204. doi:10.1371/journal.pone.0012204
57. Greer AM, Wu N, Putnam AL, Woodruff PG, Wolters P, Kinet JP, et al. Serum IgE clearance is facilitated by human FcepsilonRI internalization. *J Clin Invest* (2014) **124**:1187–98. doi:10.1172/JCI68964
58. Clanchy FI, Holloway AC, Lari R, Cameron PU, Hamilton JA. Detection and properties of the human proliferative monocyte subpopulation. *J Leukoc Biol* (2006) **79**:757–66. doi:10.1189/jlb.0905522
59. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* (1994) **1**:71–81.
60. Komano Y, Nanki T, Hayashida K, Taniguchi K, Miyasaka N. Identification of a human peripheral blood monocyte subset that differentiates into osteoclasts. *Arthritis Res Ther* (2006) **8**:R152. doi:10.1186/ar2046
61. Fingerle G, Pforte A, Passlick B, Blumenstein M, Strobel M, Ziegler-Heitbrock HWL. The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. *Blood* (1993) **82**:3170–6.
62. Horelt A, Belge KU, Steppich B, Prinz J, Ziegler-Heitbrock L. The CD14+CD16+ monocytes in erysipelas are expanded and show reduced cytokine production. *Eur J Immunol* (2002) **32**:1319–27. doi:10.1002/1521-4141(200205)32:5<1319::AID-IMMU1319>3.0.CO;2-2
63. Shalova IN, Kajiji T, Lim JY, Gomez-Pina V, Fernandez-Ruiz I, Arnalich F, et al. CD16 regulates TRIF-dependent TLR4 response in human monocytes and their subsets. *J Immunol* (2012) **188**:3584–93. doi:10.4049/jimmunol.1100244
64. Fingerle-Rowson G, Auers J, Kreuzer E, Fraunberger P, Blumenstein M, Ziegler-Heitbrock LH. Expansion of CD14+CD16+ monocytes in critically ill cardiac surgery patients. *Inflammation* (1998) **22**:367–79. doi:10.1023/A:1022316815196
65. Dayyani F, Belge KU, Frankenberger M, Mack M, Berki T, Ziegler-Heitbrock L. Mechanism of glucocorticoid-induced depletion of human CD14+CD16+ monocytes. *J Leukoc Biol* (2003) **74**:33–9. doi:10.1189/jlb.1202612
66. Korkosz M, Bukowska-Strakova K, Sadis S, Grodzicki T, Siedlar M. Monoclonal antibodies against macrophage colony-stimulating factor diminish the number of circulating intermediate and nonclassical (CD14(++)CD16(+)/CD14(+)CD16(++)) monocytes in rheumatoid arthritis patient. *Blood* (2012) **119**:5329–30. doi:10.1182/blood-2012-02-412551
67. MacDonald KP, Palmer JS, Cronau S, Seppanen E, Olver S, Raffelt NC, et al. An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-associated macrophages but does not inhibit inflammation. *Blood* (2010) **116**:3955–63. doi:10.1182/blood-2010-02-266296
68. Lenzo JC, Turner AL, Cook AD, Vlahos R, Anderson GP, Reynolds EC, et al. Control of macrophage lineage populations by CSF-1 receptor and GM-CSF in homeostasis and inflammation. *Immunol Cell Biol* (2012) **90**:429–40. doi:10.1038/icb.2011.58
69. Frankenberger M, Ekici AB, Angstwurm MW, Hoffmann H, Hofer TP, Heimbeck I, et al. A defect of CD16-positive monocytes can occur without disease. *Immunobiology* (2013) **218**:169–74. doi:10.1016/j.imbio.2012.02.013
70. Heine GH, Ortiz A, Massy ZA, Lindholm B, Wiecek A, Martinez-Castelao A, et al. Transplant, monocyte subpopulations and cardiovascular risk in chronic kidney disease. *Nat Rev Nephrol* (2012) **8**:362–9. doi:10.1038/nrneph.2012.41
71. Rogacev KS, Zawada AM, Emrich I, Seiler S, Bohm M, Fliser D, et al. Lower Apo A-I and lower HDL-C levels are associated with higher intermediate CD14++CD16+ monocyte counts that predict cardiovascular events in chronic kidney disease. *Arterioscler Thromb Vasc Biol* (2014) **34**:2120–7. doi:10.1161/ATVBAHA.114.304172

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The known unknowns of the human dendritic cell network

Mélanie Durand*[1,2] *and Elodie Segura*[1,2]

[1] *Centre de Recherche, Institut Curie, Paris, France*
[2] *INSERM U932, Paris, France*

Edited by:
Florent Ginhoux, Singapore Immunology Network, Singapore

Reviewed by:
Francesca Granucci, University of Milano-Bicocca, Italy
Kristen J. Radford, University of Queensland, Australia

***Correspondence:**
Elodie Segura, INSERM U932, Institut Curie, Pavillon Pasteur, 26 rue d'Ulm, Paris Cedex 05 75248, France
e-mail: elodie.segura@curie.fr

Dendritic cells (DCs) initiate and orient immune responses and comprise several subsets that display distinct phenotypes and properties. Most of our knowledge of DC subsets biology is based on mouse studies. In the past few years, the alignment of the human DC network with the mouse DC network has been the focus of much attention. Although comparative phenotypic and transcriptomic analysis have shown a high level of homology between mouse and human DC subsets, significant differences in phenotype and function have also been evidenced. Here, we review recent advances in our understanding of the human DC network and discuss some remaining gaps and future challenges of the human DC field.

Keywords: human, dendritic cells, antigen presentation, DC subsets, ontogeny

INTRODUCTION

Dendritic cells (DCs) have long been known to be the most efficient antigen-presenting cells. It is now well established that DCs are a heterogeneous population composed of several subsets that can be distinguished by their phenotype, location, and functional properties (1). Due to their remarkable ability to stimulate T cells, DCs have become in the past decade attractive therapeutic targets. However, most of our knowledge of DC subsets biology was gained from mouse studies, and cross-species differences could hinder the successful translation to humans of major discoveries made in the mouse. In the past few years, a number of studies have tackled the analysis of human DC subsets. In this review, we summarize recent advances and highlight some of the outstanding questions that remain to be addressed.

HOW TO DEFINE DC SUBSETS IN HUMANS?

Historically, human DC subsets have been defined based on a small number of phenotypic markers, within the population of MHC class II$^+$ lineage-negative cells. In blood, DCs have been divided into two main groups: plasmacytoid DCs (pDC) and "myeloid" or "classical" DCs (cDCs). cDCs can be further separated into two subsets that are usually referred to as BDCA1/CD1c$^+$ DCs and BDCA3/CD141$^+$ DCs (2). These three DC populations are also found in all lymphoid organs and represent resident DCs (3–6). In skin, liver, lung, and intestine, two main populations of CD1c$^+$CD1a$^+$ DCs and CD141$^+$Clec9A$^+$ DCs have been identified (7–12). Tissue DCs can migrate through the lymph to the draining lymph nodes where these migratory DCs display a mature phenotype (4, 13, 14). Additional DC subsets have been described in mucosal tissues: Langerhans cells (LCs) and CD14$^+$ DCs (15, 16) in skin and vaginal mucosa, and CD103$^-$CD172a$^+$ DCs in the intestine (10). Finally, a population of "inflammatory" DCs with a distinct phenotype can also be found in inflamed tissues (17, 18).

Although surface markers are useful for the characterization of DC subsets (**Table 1**), phenotypic analysis has proven insufficient on its own to define DC subsets. Indeed, some phenotypic markers are not specific of a given DC subset or their expression can change upon activation, potentially leading to misinterpretation. For instance, CD141 is upregulated upon activation on pDC and CD1c$^+$ DCs (19) and is also expressed by tissue CD14$^+$ DCs (20). Clec9A, which is restricted to CD141$^+$ DCs, is downregulated rapidly during DC maturation (21). Another hurdle is the promiscuous expression of some markers on macrophages and monocytes, such as CD14 or CD64. Recently, CD14$^+$CD1c^{low} cells in the skin were re-defined as macrophages (22). However, the identity of tissue CD14$^+$CD1c^{high} cells remains uncertain, we refer to these cells as CD14$^+$ DCs throughout this review.

The analysis of key DC properties can help assessing the DC identity of a potential subset. Hallmark properties include dendritic morphology, migratory capacity, and ability to stimulate naive T cells. These properties have been used to distinguish macrophages from DCs in the skin (22, 23) and inflammatory fluids (17), or monocytes from DCs in the blood (24).

Finally, gene expression signatures have emerged from transcriptomic studies and can be a useful tool to confirm DC identity, to assign a population to a known DC subset, or to define a new one. Lineage-negative CD16$^+$ blood cells were initially termed CD16$^+$ DCs, but transcriptomic analysis showed that they are a subset of monocytes (25). Similarly, 6-sulfo LacNac/Slan$^+$ blood cells were termed Slan$^+$ DCs, but comparative transcriptomic analysis identified them as a subpopulation of CD16$^+$ monocytes (24). Recently, dermal CD14$^+$CD1c^{low} cells were found to be closer in their gene expression to macrophages than to DCs (22). Transcriptomic analysis has also been used to assess the proximity of tissue DC subsets with their blood counterparts for skin (8) or intestinal DCs (10).

WHAT IS THE ONTOGENY OF HUMAN DC SUBSETS?

Addressing human DC ontogeny is challenging, but *in vitro* culture models, clinical observations, and comparative transcriptomic analysis have provided substantial insight. Human DCs are constantly replenished from bone marrow precursors as shown by the replacement of dermal DCs after hematopoietic stem cell transplantation (23) and the loss of blood DCs after bone

Table 1 | Phenotypic markers for human DC subsets.

Surface marker	pDC	Blood/ resident CD1c DC	Blood/ resident CD141 DC	Tissue/migratory CD1c CD1a DC	Tissue/migratory CD141 Clec9A DC	Tissue/ migratory CD14 DC	Inflammatory DC	Langerhans cells
HLA-DR	+	+	+	+	+	+	+	+
CD11c	–	++	+	++	+	++	++	+
CD123	+	–	–	–	–	–	–	–
BDCA2/CD303	+	–	–	–	–	–	–	–
BDCA4/CD304	+	–	–	–	–	?	?	–
Clec9A	–	–	+ Immature Low mature	–	+	–	–	–
BDCA3/CD141	– + Mature	+ Immature ++ Mature	++	+ Immature ++ Mature	++	+	?	–
XCR1	–	–	+	–	+	–	–	–
CX3CR1	?	+ Blood ? Lymphoid organs	–	+/–	–	+	?	+/–
BDCA1/CD1c	–	+	–	+	–	+	+	+
Sirp-α/CD172a	–	+	–	+	–	+	+	+
CD11b	–	– Blood + Lymphoid organs	–	+	–	+	+	+
MR/CD206	–	–	–	+	–	+	++	–
CD14	–	–	–	–	–	+	+	–
FcεRI	–	+	–	?	–	?	+	–
CD1a	–	–	–	+/–	–	–	+	++
CD64	–	+	–	+	–	?	+	?
Langerin/CD207	–	–	–	+/–	–	–	–	+
EpCAM/CD326	–	–	–	–	–	–	–	+
E-cadherin	–	–	–	–	–	–	–	+

+/–, reported in some tissues. ?, not reported.

marrow suppression induced by preparative cytotoxic therapy (22). Patients carrying mutations in *GATA2* or *IRF8* lack all blood DC subsets, consistent with a common origin (26, 27). Several lines of evidence indicate that Flt3-L is required for the generation and/or maintenance of most DC subsets: injection of Flt3-L to human volunteers increases the number of blood DC subsets (28, 29); pDCs, CD1c$^+$ DCs, and CD141$^+$ DCs equivalents can be derived *in vitro* by culturing CD34$^+$ hematopoietic precursors with Flt3-L (30–32); levels of serum Flt3-L are elevated in patients affected by mutations in *GATA2* or *IRF8* (26, 27). The importance of other cytokines in DC differentiation or homeostasis *in vivo* is unclear. Recently, a committed DC progenitor (CDP) has been identified in bone marrow and cord blood, but was absent from adult blood and tonsils (33). In an *in vitro* culture model, these CDP give rise only to pDC and cDCs, via an intermediate precursor restricted to CD1c$^+$ DCs and CD141$^+$ DCs (29, 33). This pre-cDC is present in adult bone marrow, blood, and tonsils (29). Whether pre-cDC differentiate into cDCs in the blood or lymphoid organs and tissues remains to be addressed.

The ontogeny of migratory DCs also remains to be better characterized. Of note, patients affected with a mutation in *GATA2* retain normal numbers of epidermal LC (27), showing that LC represent a distinct lineage from pDCs and cDCs. The observations that LC remained of donor origin 10 years after hand allograft and that they could proliferate *in situ* indicate that LC can self-renew in tissues (34). In addition, transcriptomic analysis shows that intestinal CD103$^-$CD172a$^+$ DCs (10) and inflammatory DCs (17) express monocyte gene signatures, suggesting that these DC subsets derive from monocytes rather than a common DC precursor.

Cross-species comparative transcriptomic analysis suggest that pDCs, CD1c$^+$ DCs, and CD141$^+$ DCs represent distinct *bona fide* lineages, as homologies have been evidenced with the well-defined mouse DC subsets pDCs, CD11b$^+$ DCs, and CD8$^+$ DCs, respectively (8, 10, 25). Regarding the molecular ontogeny, *in vitro* culture models indicate that the transcription factors E2-2 and Batf3 drive the differentiation of pDCs and CD141$^+$ DCs, respectively (35–37). Of note, Batf3 silencing in humanized mice was not sufficient to inhibit CD141$^+$ DC differentiation (37), which might be due to molecular compensation by related transcription factors as shown in Batf3-deficient mice (38). It has been proposed that CD1c$^+$ DCs depend on IRF4 based on its preferential expression in CD1c$^+$ DCs (10, 39), however this remains to be formally proven.

Another unresolved matter is the relationship of blood cDCs and their lymphoid organ and tissue counterparts. It has been suggested that blood CD1c$^+$ DCs and CD141$^+$ DCs represent a precursor form of cDC subsets (4, 8, 40), but a direct

precursor–progeny relationship remains unclear. Consistent with the idea that they are not terminally differentiated, blood CD1c$^+$ DCs and CD141$^+$ DCs become competent for cross-presentation only after activation, whereas lymphoid organ DCs cross-present without the need for activation (41). Moreover, blood CD1c$^+$ DCs retain some plasticity as they can differentiate *ex vivo* into LC-like cells, while tonsil CD1c$^+$ DCs cannot (42, 43).

IS THERE A FUNCTIONAL SPECIALIZATION OF HUMAN DC SUBSETS?

PATHOGEN RECOGNITION

Among the variety of pathogen-recognition receptors, TLR expression by DC subsets (either mRNA or protein expression) has been the most studied. pDC express TLR1, TLR6, TLR7, TLR9, and TLR10, resident CD1c$^+$ DCs express TLR1, TLR2, TLR4, TLR5, TLR6, and TLR8, and resident CD141$^+$ DCs express TLR1, TLR3, TLR6, TLR8, and TLR10 (3, 5, 44–46). Skin LC express TLR1, TLR2, TLR3, TLR6, TLR7 and vaginal mucosa LC express TLR8 in addition (15, 47, 48), skin and vaginal mucosa CD1a$^+$ DCs express TLR6 and TLR8 while the expression of other TLR is less clear, and skin and vaginal mucosa CD14$^+$ DCs express TLR1, TLR2, TLR4, TLR6, and TLR8 (15, 49). C-type lectin receptors are also important pathogen-recognition receptors, some of which have been reported to be differentially expressed by DC subsets by transcriptomic analysis (17, 49–52). Receptors whose differential expression among DC subsets has been confirmed at protein level include Clec9A on CD141$^+$ DCs, BDCA2/CD303 on pDC, ClecSF14/CD301 on CD1c$^+$ DCs, Langerin/CD207 on LC, and Clec10a and LOX-1 on CD14$^+$ DCs (5, 19, 49, 53, 54).

Differential expression of pathogen-recognition receptors can confer functional specialization to DC subsets for the response to pathogens (46, 55, 56) or vaccines (57).

Much work remains to be done to characterize the expression pattern of intracellular pathogen-recognition receptors in resident and migratory DC subsets. It has been reported so far that vaginal mucosa and skin LC, CD1a$^+$ DCs, and CD14$^+$ DCs, all express MDA-5, while only CD14$^+$ DCs express RIG-I (15, 49).

CYTOKINE SECRETION

Blood and lymphoid organ pDC have long been known to be the best producers of type I interferon (58–60). CD141$^+$ DCs from blood and from humanized mice spleen have also been reported to be the most potent for type I interferon production after TLR3 stimulation (5, 61). More recently, blood and liver CD141$^+$ DCs were shown to selectively secrete type III interferon after activation with TLR3 ligand or Hepatitis C virus (56, 62, 63).

Because cytokine secretion by a given DC subset vary depending on the stimulus used (45), it can be difficult to determine *bona fide* specialization for cytokine secretion. Accumulating evidence indicates that blood CD1c$^+$ DCs are the best producers of IL-12p70, as shown by stimulation with TLR2, TLR3, and TLR8 ligands (40, 45, 46). Whether CD1c$^+$ DCs from tissues are also specialized for IL-12p70 secretion needs to be confirmed. Indeed, no IL-12p70 secretion could be detected after stimulation of skin DC subsets (8) or intestinal CD1c$^+$ DCs (11) with several TLR-ligands. Intestinal and lung CD1c$^+$ DCs are also the best producers of IL-23 after TLR8 stimulation or *Aspergillus fumigatus* exposure, respectively (11, 39). CD1c$^+$ DCs from skin, intestine, and blood are also the most potent producers of IL-10 in response to several TLR-ligands (8, 11, 64). Skin LC and CD1a$^+$ DCs have been reported to be better producers of IL-15 than skin CD14$^+$ DCs (53, 65), but IL-15 secretion by other DC subsets has not been analyzed yet.

CROSS-PRESENTATION AND CD8 T-CELL RESPONSES

Numerous studies have shown that blood and lymphoid organ DC subsets can all cross-present efficiently various forms of antigen (66). Spleen, lymph node, and tonsil CD1c$^+$ and CD141$^+$ DC subsets are equally potent for cross-presenting soluble antigens, without the need for activation (4, 41, 44). When stimulated with TLR-ligands that can activate both subsets, blood CD1c$^+$ and CD141$^+$ DCs also display similar efficiency for cross-presentation (40, 44, 67). However, lymphoid organ and activated blood CD141$^+$ DCs appear to be more efficient for the cross-presentation of dead cell-derived antigen (5, 41, 68), which might be due to their selective expression of necrotic cell receptors such as Clec9A. Blood CD141$^+$ DCs were also more efficient than CD1c$^+$ DCs for cross-presentation of antigens delivered to late endocytic compartments via CD205 targeting, but were equally potent after antigen delivery to early endocytic compartments via CD40 (69).

Blood and lymphoid organ pDCs cross-present efficiently soluble (41, 44, 67, 70, 71), viral (71–74), cell-associated antigen (67, 75), or antigen targeted to surface receptors such as CD40, DCIR, CD205, BDCA2/CD303, or CD32 (67, 69, 76).

The ability of tissue DCs to cross-present is less well characterized. Skin CD1a$^+$ DCs and LC have been shown to cross-present when purified from skin or skin-draining lymph nodes (4, 77), however, a subsequent study reported that skin CD141$^+$Clec9A$^+$ DCs are the most efficient for cross-presentation compared to other skin DC subsets (8). Skin LC also cross-present antigen targeted through DCIR (76). Both CD1c$^+$ DCs and CD141$^+$ DCs from the lung of humanized mice can cross-present (9), but these results need to be confirmed with DCs directly purified from human lung. The cross-presentation capacity of migratory DCs from other tissues and of inflammatory DCs remains to be analyzed. In addition, which DC subsets cross-present *in vivo* in a physiological situation is a challenging question that is still unaddressed.

Skin LC and CD1a$^+$ DCs induce the differentiation of cytotoxic T lymphocytes (CTL) more efficiently than skin CD14$^+$ DCs, through the secretion of IL-15 (53, 65). Activated LC also express higher levels of CD70, which promotes CTL differentiation (55, 77). Blood-activated CD1c$^+$ DCs induce higher expression of granzymes B and K by CTL than activated CD141$^+$ DCs, due to the selective secretion of IL-12p70 (40). Whether this specialization also applies to lymphoid organ and tissue CD1c$^+$ DCs remains to be confirmed.

CD4 T CELLS RESPONSES

The vast majority of studies have analyzed the ability of isolated DC subsets to stimulate and polarize allogeneic naive CD4 T cells. Blood, lymph node, or lung CD1c$^+$ DCs and CD141$^+$ DCs are equally competent for Th1 polarization, either without activation

(4, 78) or after exposure to influenza virus (78) or *A. fumigatus* (39). By contrast, blood and lung CD141$^+$ DCs have been found to be more potent inducers of Th2 polarization compared to CD1c$^+$ DCs, with or without activation, due to the selective expression of OX40-L (78). Lung CD1c$^+$ DCs exposed to *A. fumigatus* are more potent than CD141$^+$ DCs for Th-17 polarization due to their secretion of IL-23 (39), however both intestinal CD1c$^+$ DCs and CD141$^+$ DCs are equally able to induce Th-17 polarization (10). Blood pDC can induce Th1 polarization after activation with CD40-L, influenza virus, or Sendai virus (60, 79), but induce Th2 polarization through OX40-L after activation with IL3 (79). Whether these observations also apply to lymphoid organ pDC, and whether pDC can induce Th-17 polarization when adequately activated remains to be addressed.

Skin DC subsets have been proposed to be specialized for CD4 T-cell polarization, LC, and CD1a$^+$ DCs being especially potent for Th2 polarization while CD14$^+$ DCs mainly induce T follicular helper (Tfh) cells (53). This specialization is conserved after skin DC migration to draining lymph nodes (4). The molecular mechanism underlying this functional specialization remains unclear. Moreover, vaginal mucosa LC and CD1a$^+$ DCs preferentially induce Th2 polarization, while vaginal mucosa CD14$^+$ DCs are better inducers of Th1 (15). The ability of vaginal mucosa DCs to induce Tfh has not been analyzed. In addition, skin LC have

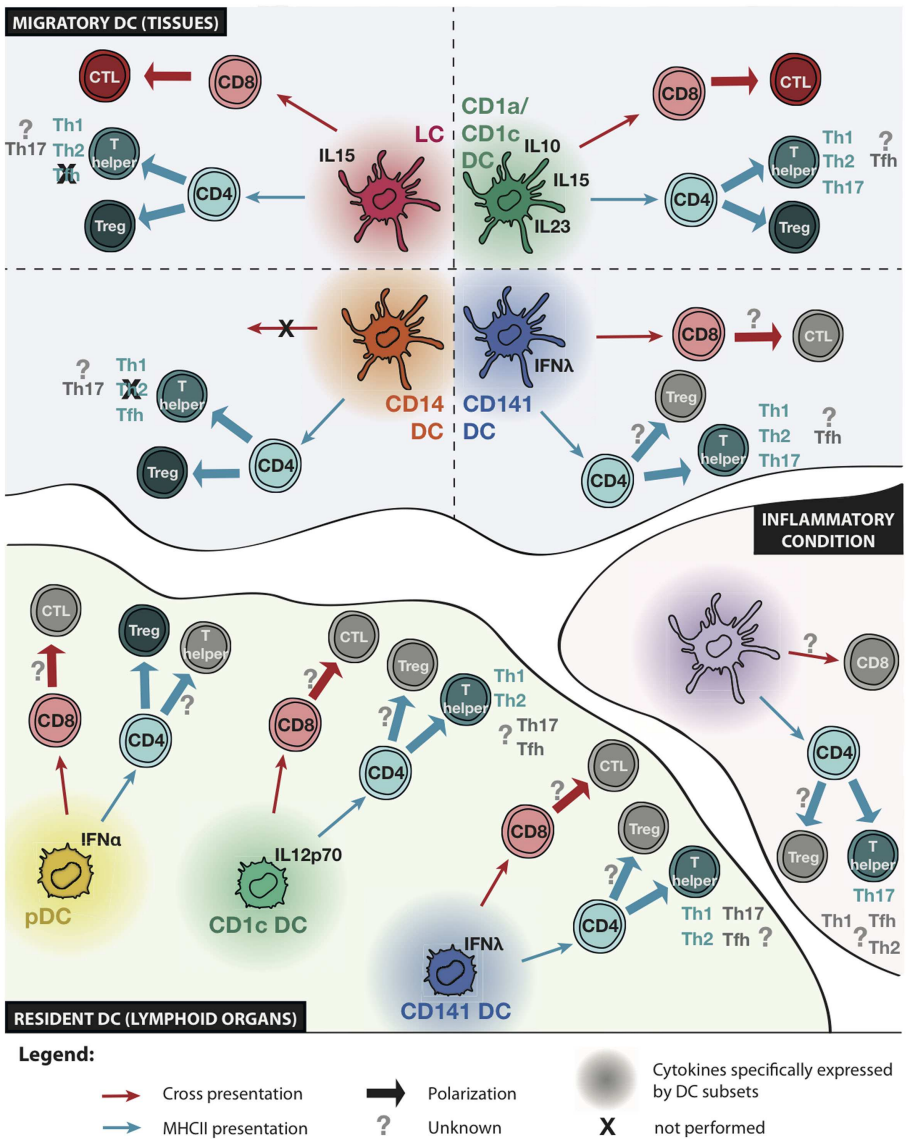

FIGURE 1 | Functional specialization of human DC subsets. Schematic representation of known and unknown functional specialization of migratory and resident DC subsets, and inflammatory DCs. Cytokines specifically expressed by a given DC subset are indicated. The ability to present antigens to CD8 or CD4 T cells is represented with red or blue arrows, respectively. The ability of DC subsets to induce cytotoxic T lymphocyte (CTL) differentiation, regulatory T (Treg), or helper T (Th) cell polarization is indicated. Question marks indicate unknown functions and crosses indicate functions that are not performed by a given DC subset.

been found to be more potent than other skin DCs for the induction of IL22-secreting CD4 T cells (80, 81), while both vaginal mucosa LC and CD1a$^+$ DCs are equally competent (15).

Inflammatory DCs isolated from rheumatoid arthritis synovial fluid and from tumor ascites preferentially induce Th-17 polarization through the secretion of Th-17 polarizing cytokines IL-6, IL-23, TGFβ, and IL1-β (17). Inflammatory DCs from tumor ascites also efficiently stimulate autologous effector CD4 T cells to secrete IL-17 (17). The CD4 T-cell responses induced by inflammatory DCs from other inflammatory environments remain to be investigated.

Finally, several DC subsets were shown to induce Treg: dermal CD14$^+$ DCs (20), intestinal CD1c$^+$ DCs and CD103$^-$CD172a$^+$ DCs (10), tonsil pDC activated with IL3 or TLR-ligands (82), and bacteria-exposed skin LC (83). In addition, liver DCs (7) and TLR4-activated oral mucosa DCs (84) were proposed to promote Treg induction through the secretion of IL-10, but whether one subset is more potent for Treg induction has not been analyzed. Blood *Escherichia coli*-activated CD1c$^+$ DCs have also been proposed to inhibit CD4 T-cell proliferation through IL-10 secretion (64). In addition, skin LC, but not dermal CD1a$^+$ DCs and CD14$^+$ DCs, have been shown to stimulate the proliferation of autologous skin-resident memory Treg (85).

Collectively, these results suggest that some CD4 T-cell responses are the consequence of subset-intrinsic specialization, while others are more dependent on signals from the environment or on tissue imprinting (**Figure 1**). The clearer observations so far are the specialization of CD141$^+$ DCs for Th2 polarization and of CD14$^+$ DCs for Tfh polarization (both findings would need to be confirmed with DCs from other tissues), and of skin and vaginal mucosal LC and CD1a$^+$ DCs for Th2 and Th22 polarization. However, this specialization might be tissue-dependent as lung CD1c$^+$CD1a$^+$ DCs are not potent inducers of Th2 polarization.

CONCLUSION

Despite the technical challenges inherent to human DC work, significant progress has been made in the past few years in the characterization of human DC subsets. Important issues that will need further exploration include the ability of DC subsets to stimulate effector and memory T cells, the interplay between DC subsets, and the *in vivo* confirmation of functional specializations observed *ex vivo*. These could be achieved by the use of humanized mice models, the analysis of relevant pathological situations, or the study of patients with mutations in relevant genes.

This knowledge will be instrumental in the design of novel vaccines and DC-based immunotherapies.

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REFERENCES

1. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* (2013) **31**:563–604. doi:10.1146/annurev-immunol-020711-074950
2. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* (2010) **116**(16):e74–80. doi:10.1182/blood-2010-02-258558
3. Lindstedt M, Lundberg K, Borrebaeck CA. Gene family clustering identifies functionally associated subsets of human in vivo blood and tonsillar dendritic cells. *J Immunol* (2005) **175**(8):4839–46. doi:10.4049/jimmunol.175.8.4839
4. Segura E, Valladeau-Guilemond J, Donnadieu MH, Sastre-Garau X, Soumelis V, Amigorena S. Characterization of resident and migratory dendritic cells in human lymph nodes. *J Exp Med* (2012) **209**(4):653–60. doi:10.1084/jem.20111457
5. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* (2010) **207**(6):1247–60. doi:10.1084/jem.20092140
6. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen JL, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**(6):1261–71. doi:10.1084/jem.20092618
7. Bamboat ZM, Stableford JA, Plitas G, Burt BM, Nguyen HM, Welles AP, et al. Human liver dendritic cells promote T cell hyporesponsiveness. *J Immunol* (2009) **182**(4):1901–11. doi:10.4049/jimmunol.0803404
8. Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, et al. Human tissues contain CD141(hi) cross-presenting dendritic cells with functional homology to mouse CD103(+) nonlymphoid dendritic cells. *Immunity* (2012) **37**(1):60–73. doi:10.1016/j.immuni.2012.04.012
9. Yu CI, Becker C, Wang Y, Marches F, Helft J, Leboeuf M, et al. Human CD1c(+) dendritic cells drive the differentiation of CD103(+) CD8(+) mucosal effector T cells via the cytokine TGF-beta. *Immunity* (2013) **38**(4):818–30. doi:10.1016/j.immuni.2013.03.004
10. Watchmaker PB, Lahl K, Lee M, Baumjohann D, Morton J, Kim SJ, et al. Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. *Nat Immunol* (2014) **15**(1):98–108. doi:10.1038/ni.2768
11. Dillon SM, Rogers LM, Howe R, Hostetler LA, Buhrman J, McCarter MD, et al. Human intestinal lamina propria CD1c+ dendritic cells display an activated phenotype at steady state and produce IL-23 in response to TLR7/8 stimulation. *J Immunol* (2010) **184**(12):6612–21. doi:10.4049/jimmunol.1000041
12. Demedts IK, Brusselle GG, Vermaelen KY, Pauwels RA. Identification and characterization of human pulmonary dendritic cells. *Am J Respir Cell Mol Biol* (2005) **32**(3):177–84. doi:10.1165/rcmb.2004-0279OC
13. Morandi B, Bonaccorsi I, Mesiti M, Conte R, Carrega P, Costa G, et al. Characterization of human afferent lymph dendritic cells from seroma fluids. *J Immunol* (2013) **191**(9):4858–66. doi:10.4049/jimmunol.1300760
14. Angel CE, Chen CJ, Horlacher OC, Winkler S, John T, Browning J, et al. Distinctive localization of antigen-presenting cells in human lymph nodes. *Blood* (2009) **113**(6):1257–67. doi:10.1182/blood-2008-06-165266
15. Duluc D, Gannevat J, Anguiano E, Zurawski S, Carley M, Boreham M, et al. Functional diversity of human vaginal APC subsets in directing T-cell responses. *Mucosal Immunol* (2013) **6**(3):626–38. doi:10.1038/mi.2012.104
16. Nestle FO, Zheng XG, Thompson CB, Turka LA, Nickoloff BJ. Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. *J Immunol* (1993) **151**(11):6535–45.
17. Segura E, Touzot M, Bohineust A, Cappuccio A, Chiocchia G, Hosmalin A, et al. Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity* (2013) **38**(2):336–48. doi:10.1016/j.immuni.2012.10.018
18. Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Whynot J, Novitskaya I, Cardinale I, et al. Major differences in inflammatory dendritic cells and their products distinguish atopic dermatitis from psoriasis. *J Allergy Clin Immunol* (2007) **119**(5):1210–7. doi:10.1016/j.jaci.2007.03.006
19. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* (2000) **165**(11):6037–46. doi:10.4049/jimmunol.165.11.6037
20. Chu CC, Ali N, Karagiannis P, Di Meglio P, Skowera A, Napolitano L, et al. Resident CD141 (BDCA3)+ dendritic cells in human skin produce IL-10 and induce regulatory T cells that suppress skin inflammation. *J Exp Med* (2012) **209**(5):935–45. doi:10.1084/jem.20112583

21. Schreibelt G, Klinkenberg LJ, Cruz LJ, Tacken PJ, Tel J, Kreutz M, et al. The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-)presentation by human blood BDCA3+ myeloid dendritic cells. *Blood* (2012) **119**(10):2284–92. doi:10.1182/blood-2011-08-373944
22. McGovern N, Schlitzer A, Gunawan M, Jardine L, Shin A, Poyner E, et al. Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages. *Immunity* (2014) **41**(3):465–77. doi:10.1016/j.immuni.2014.08.006
23. Haniffa M, Ginhoux F, Wang XN, Bigley V, Abel M, Dimmick I, et al. Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. *J Exp Med* (2009) **206**(2):371–85. doi:10.1084/jem.20081633
24. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* (2010) **33**(3):375–86. doi:10.1016/j.immuni.2010.08.012
25. Robbins SH, Walzer T, Dembele D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* (2008) **9**(1):R17. doi:10.1186/gb-2008-9-1-r17
26. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. IRF8 mutations and human dendritic-cell immunodeficiency. *N Engl J Med* (2011) **365**(2):127–38. doi:10.1056/NEJMoa1100066
27. Bigley V, Haniffa M, Doulatov S, Wang XN, Dickinson R, McGovern N, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. *J Exp Med* (2011) **208**(2):227–34. doi:10.1084/jem.20101459
28. Pulendran B, Banchereau J, Burkeholder S, Kraus E, Guinet E, Chalouni C, et al. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J Immunol* (2000) **165**(1):566–72. doi:10.4049/jimmunol.165.1.566
29. Breton G, Lee J, Zhou YJ, Schreiber JJ, Keler T, Puhr S, et al. Circulating precursors of human CD1c+ and CD141+ dendritic cells. *J Exp Med* (2015) **212**(3):401–13. doi:10.1084/jem.20141441
30. Chen W, Antonenko S, Sederstrom JM, Liang X, Chan AS, Kanzler H, et al. Thrombopoietin cooperates with FLT3-ligand in the generation of plasmacytoid dendritic cell precursors from human hematopoietic progenitors. *Blood* (2004) **103**(7):2547–53. doi:10.1182/blood-2003-09-3058
31. Blom B, Ho S, Antonenko S, Liu YJ. Generation of interferon alpha-producing predendritic cell (Pre-DC)2 from human CD34(+) hematopoietic stem cells. *J Exp Med* (2000) **192**(12):1785–96. doi:10.1084/jem.192.12.1785
32. Proietto AI, Mittag D, Roberts AW, Sprigg N, Wu L. The equivalents of human blood and spleen dendritic cell subtypes can be generated in vitro from human CD34(+) stem cells in the presence of fms-like tyrosine kinase 3 ligand and thrombopoietin. *Cell Mol Immunol* (2012) **9**(6):446–54. doi:10.1038/cmi.2012.48
33. Lee J, Breton G, Oliveira TY, Zhou YJ, Aljoufi A, Puhr S, et al. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. *J Exp Med* (2015) **212**(3):385–99. doi:10.1084/jem.20141442
34. Kanitakis J, Morelon E, Petruzzo P, Badet L, Dubernard JM. Self-renewal capacity of human epidermal Langerhans cells: observations made on a composite tissue allograft. *Exp Dermatol* (2011) **20**(2):145–6. doi:10.1111/j.1600-0625.2010.01146.x
35. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* (2008) **135**(1):37–48. doi:10.1016/j.cell.2008.09.016
36. Nagasawa M, Schmidlin H, Hazekamp MG, Schotte R, Blom B. Development of human plasmacytoid dendritic cells depends on the combined action of the basic helix-loop-helix factor E2-2 and the Ets factor Spi-B. *Eur J Immunol* (2008) **38**(9):2389–400. doi:10.1002/eji.200838470
37. Poulin LF, Reyal Y, Uronen-Hansson H, Schraml B, Sancho D, Murphy KM, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and non-lymphoid tissues. *Blood* (2012) **119**(25):6052–62. doi:10.1182/blood-2012-01-406967
38. Tussiwand R, Lee WL, Murphy TL, Mashayekhi M, Kc W, Albring JC, et al. Compensatory dendritic cell development mediated by BATF-IRF interactions. *Nature* (2012) **490**(7421):502–7. doi:10.1038/nature11531
39. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* (2013) **38**(5):970–83. doi:10.1016/j.immuni.2013.04.011
40. Nizzoli G, Krietsch J, Weick A, Steinfelder S, Facciotti F, Gruarin P, et al. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T cell responses. *Blood* (2013) **122**(6):932–42. doi:10.1182/blood-2013-04-495424
41. Segura E, Durand M, Amigorena S. Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. *J Exp Med* (2013) **210**(5):1035–47. doi:10.1084/jem.20121103
42. Martinez-Cingolani C, Grandclaudon M, Jeanmougin M, Jouve M, Zollinger R, Soumelis V. Human blood BDCA-1 dendritic cells differentiate into Langerhans-like cells with thymic stromal lymphopoietin and TGF-beta. *Blood* (2014) **124**(15):2411–20. doi:10.1182/blood-2014-04-568311
43. Milne P, Bigley V, Gunawan M, Haniffa M, Collin M. CD1c+ blood dendritic cells have Langerhans cell potential. *Blood* (2014) **125**(3):470–3. doi:10.1182/blood-2014-08-593582
44. Mittag D, Proietto AI, Loudovaris T, Mannering SI, Vremec D, Shortman K, et al. Human dendritic cell subsets from spleen and blood are similar in phenotype and function but modified by donor health status. *J Immunol* (2011) **186**(11):6207–17. doi:10.4049/jimmunol.1002632
45. Hemont C, Neel A, Heslan M, Braudeau C, Josien R. Human blood mDC subsets exhibit distinct TLR repertoire and responsiveness. *J Leukoc Biol* (2013) **93**(4):599–609. doi:10.1189/jlb.0912452
46. Jin JO, Zhang W, Du JY, Yu Q. BDCA1-positive dendritic cells (DCs) represent a unique human myeloid DC subset that induces innate and adaptive immune responses to *Staphylococcus aureus* infection. *Infect Immun* (2014) **82**(11):4466–76. doi:10.1128/IAI.01851-14
47. van der Aar AM, Sylva-Steenland RM, Bos JD, Kapsenberg ML, de Jong EC, Teunissen MB. Loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. *J Immunol* (2007) **178**(4):1986–90. doi:10.4049/jimmunol.178.4.1986
48. Flacher V, Bouschbacher M, Verronese E, Massacrier C, Sisirak V, Berthier-Vergnes O, et al. Human Langerhans cells express a specific TLR profile and differentially respond to viruses and Gram-positive bacteria. *J Immunol* (2006) **177**(11):7959–67. doi:10.4049/jimmunol.177.11.7959
49. Duluc D, Banchereau R, Gannevat J, Thompson-Snipes L, Blanck JP, Zurawski S, et al. Transcriptional fingerprints of antigen-presenting cell subsets in the human vaginal mucosa and skin reflect tissue-specific immune microenvironments. *Genome Med* (2014) **6**(11):98. doi:10.1186/s13073-014-0098-y
50. Santegoets SJ, Gibbs S, Kroeze K, van de Ven R, Scheper RJ, Borrebaeck CA, et al. Transcriptional profiling of human skin-resident Langerhans cells and CD1a+ dermal dendritic cells: differential activation states suggest distinct functions. *J Leukoc Biol* (2008) **84**(1):143–51. doi:10.1189/jlb.1107750
51. Lundberg K, Albrekt AS, Nelissen I, Santegoets S, de Gruijl TD, Gibbs S, et al. Transcriptional profiling of human dendritic cell populations and models – unique profiles of in vitro dendritic cells and implications on functionality and applicability. *PLoS One* (2013) **8**(1):e52875. doi:10.1371/journal.pone.0052875
52. Harman AN, Bye CR, Nasr N, Sandgren KJ, Kim M, Mercier SK, et al. Identification of lineage relationships and novel markers of blood and skin human dendritic cells. *J Immunol* (2013) **190**(1):66–79. doi:10.4049/jimmunol.1200779
53. Klechevsky E, Morita R, Liu M, Cao Y, Coquery S, Thompson-Snipes L, et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity* (2008) **29**(3):497–510. doi:10.1016/j.immuni.2008.07.013
54. Lundberg K, Rydnert F, Greiff L, Lindstedt M. Human blood dendritic cell subsets exhibit discriminative pattern recognition receptor profiles. *Immunology* (2014) **142**(2):279–88. doi:10.1111/imm.12252
55. van der Aar AM, de Groot R, Sanchez-Hernandez M, Taanman EW, van Lier RA, Teunissen MB, et al. Cutting edge: virus selectively primes human Langerhans cells for CD70 expression promoting CD8+ T cell responses. *J Immunol* (2011) **187**(7):3488–92. doi:10.4049/jimmunol.1101105
56. Zhang S, Kodys K, Li K, Szabo G. Human type 2 myeloid dendritic cells produce interferon-lambda and amplify interferon-alpha in response to hepatitis C virus infection. *Gastroenterology* (2013) **144**(2):414–25.e7. doi:10.1053/j.gastro.2012.10.034
57. Banchereau R, Baldwin N, Cepika AM, Athale S, Xue Y, Yu CI, et al. Transcriptional specialization of human dendritic cell subsets in response to microbial vaccines. *Nat Commun* (2014) **5**:5283. doi:10.1038/ncomms6283

58. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* (1999) **5**(8):919–23. doi:10.1038/11360
59. Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science* (1999) **284**(5421):1835–7. doi:10.1126/science.284.5421.1835
60. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat Immunol* (2000) **1**(4):305–10. doi:10.1038/79747
61. Meixlsperger S, Leung CS, Ramer PC, Pack M, Vanoaica LD, Breton G, et al. CD141+ dendritic cells produce prominent amounts of IFN-alpha after dsRNA recognition and can be targeted via DEC-205 in humanized mice. *Blood* (2013) **121**(25):5034–44. doi:10.1182/blood-2012-12-473413
62. Lauterbach H, Bathke B, Gilles S, Traidl-Hoffmann C, Luber CA, Fejer G, et al. Mouse CD8alpha+ DCs and human BDCA3+ DCs are major producers of IFN-lambda in response to poly IC. *J Exp Med* (2010) **207**(12):2703–17. doi:10.1084/jem.20092720
63. Yoshio S, Kanto T, Kuroda S, Matsubara T, Higashitani K, Kakita N, et al. Human blood dendritic cell antigen 3 (BDCA3)(+) dendritic cells are a potent producer of interferon-lambda in response to hepatitis C virus. *Hepatology* (2013) **57**(5):1705–15. doi:10.1002/hep.26182
64. Kassianos AJ, Hardy MY, Ju X, Vijayan D, Ding Y, Vulink AJ, et al. Human CD1c (BDCA-1)+ myeloid dendritic cells secrete IL-10 and display an immunoregulatory phenotype and function in response to *Escherichia coli*. *Eur J Immunol* (2012) **42**(6):1512–22. doi:10.1002/eji.201142098
65. Banchereau J, Thompson-Snipes L, Zurawski S, Blanck JP, Cao Y, Clayton S, et al. The differential production of cytokines by human Langerhans cells and dermal CD14(+) DCs controls CTL priming. *Blood* (2012) **119**(24):5742–9. doi:10.1182/blood-2011-08-371245
66. Segura E, Amigorena S. Cross-presentation by human dendritic cell subsets. *Immunol Lett* (2014) **158**(1–2):73–8. doi:10.1016/j.imlet.2013.12.001
67. Tel J, Schreibelt G, Sittig SP, Mathan TS, Buschow SI, Cruz LJ, et al. Human plasmacytoid dendritic cells efficiently cross-present exogenous Ags to CD8+ T cells despite lower Ag uptake than myeloid dendritic cell subsets. *Blood* (2013) **121**(3):459–67. doi:10.1182/blood-2012-06-435644
68. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, Ventre E, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* (2010) **207**(6):1283–92. doi:10.1084/jem.20100223
69. Cohn L, Chatterjee B, Esselborn F, Smed-Sorensen A, Nakamura N, Chalouni C, et al. Antigen delivery to early endosomes eliminates the superiority of human blood BDCA3+ dendritic cells at cross presentation. *J Exp Med* (2013) **210**(5):1049–63. doi:10.1084/jem.20121251
70. Aspord C, Leloup C, Reche S, Plumas J. pDCs efficiently process synthetic long peptides to induce functional virus- and tumour-specific T-cell responses. *Eur J Immunol* (2014) **44**(10):2880–92. doi:10.1002/eji.201444588
71. Hoeffel G, Ripoche AC, Matheoud D, Nascimbeni M, Escriou N, Lebon P, et al. Antigen crosspresentation by human plasmacytoid dendritic cells. *Immunity* (2007) **27**(3):481–92. doi:10.1016/j.immuni.2007.07.021
72. Di Pucchio T, Chatterjee B, Smed-Sorensen A, Clayton S, Palazzo A, Montes M, et al. Direct proteasome-independent cross-presentation of viral antigen by plasmacytoid dendritic cells on major histocompatibility complex class I. *Nat Immunol* (2008) **9**(5):551–7. doi:10.1038/ni.1602
73. Lui G, Manches O, Angel J, Molens JP, Chaperot L, Plumas J. Plasmacytoid dendritic cells capture and cross-present viral antigens from influenza-virus exposed cells. *PLoS One* (2009) **4**(9):e7111. doi:10.1371/journal.pone.0007111
74. Zhang X, Casartelli N, Lemoine S, Mozeleski B, Azria E, Le Ray C, et al. Plasmacytoid dendritic cells engagement by influenza vaccine as a surrogate strategy for driving T-helper type 1 responses in human neonatal settings. *J Infect Dis* (2014) **210**(3):424–34. doi:10.1093/infdis/jiu103
75. Guillerme JB, Boisgerault N, Roulois D, Menager J, Combredet C, Tangy F, et al. Measles virus vaccine-infected tumor cells induce tumor antigen cross-presentation by human plasmacytoid dendritic cells. *Clin Cancer Res* (2013) **19**(5):1147–58. doi:10.1158/1078-0432.CCR-12-2733
76. Klechevsky E, Flamar AL, Cao Y, Blanck JP, Liu M, O'Bar A, et al. Cross-priming CD8+ T cells by targeting antigens to human dendritic cells through DCIR. *Blood* (2010) **116**(10):1685–97. doi:10.1182/blood-2010-01-264960
77. Polak ME, Newell L, Taraban VY, Pickard C, Healy E, Friedmann PS, et al. CD70-CD27 interaction augments CD8+ T-cell activation by human epidermal Langerhans cells. *J Invest Dermatol* (2012) **132**(6):1636–44. doi:10.1038/jid.2012.26
78. Yu CI, Becker C, Metang P, Marches F, Wang Y, Toshiyuki H, et al. Human CD141+ dendritic cells induce CD4+ T cells to produce type 2 cytokines. *J Immunol* (2014) **193**(9):4335–43. doi:10.4049/jimmunol.1401159
79. Ito T, Amakawa R, Inaba M, Hori T, Ota M, Nakamura K, et al. Plasmacytoid dendritic cells regulate Th cell responses through OX40 ligand and type I IFNs. *J Immunol* (2004) **172**(7):4253–9. doi:10.4049/jimmunol.172.7.4253
80. Fujita H, Nograles KE, Kikuchi T, Gonzalez J, Carucci JA, Krueger JG. Human Langerhans cells induce distinct IL-22-producing CD4+ T cells lacking IL-17 production. *Proc Natl Acad Sci U S A* (2009) **106**(51):21795–800. doi:10.1073/pnas.0911472106
81. Penel-Sotirakis K, Simonazzi E, Peguet-Navarro J, Rozieres A. Differential capacity of human skin dendritic cells to polarize CD4+ T cells into IL-17, IL-21 and IL-22 producing cells. *PLoS One* (2012) **7**(11):e45680. doi:10.1371/journal.pone.0045680
82. Palomares O, Ruckert B, Jartti T, Kucuksezer UC, Puhakka T, Gomez E, et al. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. *J Allergy Clin Immunol* (2012) **129**(2):510–20, 520.e1–9. doi:10.1016/j.jaci.2011.09.031
83. van der Aar AM, Picavet DI, Muller FJ, de Boer L, van Capel TM, Zaat SA, et al. Langerhans cells favor skin flora tolerance through limited presentation of bacterial antigens and induction of regulatory T cells. *J Invest Dermatol* (2013) **133**(5):1240–9. doi:10.1038/jid.2012.500
84. Allam JP, Peng WM, Appel T, Wenghoefer M, Niederhagen B, Bieber T, et al. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. *J Allergy Clin Immunol* (2008) **121**(2):368–74.e1. doi:10.1016/j.jaci.2007.09.045
85. Seneschal J, Clark RA, Gehad A, Baecher-Allan CM, Kupper TS. Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. *Immunity* (2012) **36**(5):873–84. doi:10.1016/j.immuni.2012.03.018

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A systematic approach to identify markers of distinctly activated human macrophages

Bayan Sudan[1], *Mark A. Wacker*[1], *Mary E. Wilson*[1,2] *and Joel W. Graff*[1,2]*

[1] *Infectious Diseases Division, Department of Internal Medicine, University of Iowa, Iowa City, IA, USA,* [2] *Iowa City VA Medical Center, Iowa City, IA, USA*

Polarization has been a useful concept for describing activated macrophage phenotypes and gene expression profiles. However, macrophage activation status within tumors and other settings are often inferred based on only a few markers. Complicating matters for relevance to human biology, many macrophage activation markers have been best characterized in mice and sometimes are not similarly regulated in human macrophages. To identify novel markers of activated human macrophages, gene expression profiles for human macrophages of a single donor subjected to 33 distinct activating conditions were obtained and a set of putative activation markers were subsequently evaluated in macrophages from multiple donors using integrated fluidic circuit (IFC)-based RT-PCR. Using unsupervised hierarchical clustering of the microarray screen, highly altered transcripts (>4-fold change in expression) sorted the macrophage transcription profiles into two major and 13 minor clusters. Among the 1874 highly altered transcripts, over 100 were uniquely altered in one major or two related minor clusters. IFC PCR-derived data confirmed the microarray results and determined the kinetics of expression of potential macrophage activation markers. Transcripts encoding chemokines, cytokines, and cell surface were prominent in our analyses. The activation markers identified by this study could be used to better characterize tumor-associated macrophages from biopsies as well as other macrophage populations collected from human clinical samples.

Keywords: human macrophages, activation markers, microarray, integrated fluidic circuit RT-PCR, macrophage polarization

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Martin Guilliams, VIB Inflammation Research Center, Belgium

Reviewed by:
Elodie Segura, Institut Curie, France
Sam Basta, Queen's University, Canada

***Correspondence:**
Joel W. Graff, Infectious Diseases Division, Department of Internal Medicine, University of Iowa, 400 EMRB, Iowa City, IA 52242, USA
joel-graff@uiowa.edu

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Introduction

Macrophages assume critical roles in almost every tissue and disease state through their ability to assume distinct functional capacities in different microenvironments. Macrophages respond to a variety of external stimuli to assume different polarized activation states. Distinctly polarized macrophages, modeled *in vitro* using specific activating conditions, can be defined by functional attributes such as microbicidal activity, and by unique gene expression profiles. An early study contrasting functional and gene expression differences between IFNγ- and IL-4-treated macrophages proposed that the latter phenotype be described as alternative activation (1), a very different macrophage phenotype from IFNγ- or classically activated macrophages. Since that time, many additional polarized macrophage types, induced by different stimuli, have been proposed.

Several competing systems have been proposed in an attempt to provide a framework that describes the complexity of macrophage polarization. The first system describes macrophage phenotypes as a linear continuum with M1 (classically activated) and M2 (alternatively activated) macrophages at opposite ends (2, 3). The second system describes macrophage phenotypes as a spectrum akin to a color wheel, with classically activated, wound healing, and regulatory macrophages used as examples of unique polarized phenotypes that do not fit well within a linear continuum (4). A modified version of the M1–M2 system acknowledged the diversity of macrophage phenotypes with descriptions such as M1a, M1b, M2a, M2b, and M2c (5, 6). Additions to the M1–M2 nomenclature system have proposed naming macrophages differentiated in the presence of CXCL4 as "M4" (7) and IL-17-treated macrophages "M17" (8). To standardize the burgeoning descriptions of polarized macrophage types, it has been suggested that the activation condition be defined in the name of the polarized macrophage [M(IL-4), M(IL-10), M(LPS), M(IFNγ), and so forth (9)]. To preserve clarity, we have employed this descriptive nomenclature system to describe the activated macrophages in the current report (**Table 1**).

Macrophages are often very abundant within tumors (12, 13). There is evidence that macrophages can promote tumorigenesis, tumor growth, and metastasis (14). Despite macrophage pro-tumor activities, tumor-associated macrophages (TAMs) display a wide range of phenotypic diversity within a tumor due to ontogeny, activation signals, and localization (15). The plasticity of macrophage phenotypes is well known (16, 17) and this characteristic has provided a therapeutic target whereby macrophages are encouraged to switch functionally from pro-tumor to anti-tumor. Clinical approaches that modify macrophage activation in this way include blockade of M-CSF, low-dose irradiation, and combinational therapies (18–21). What is lacking is a thoroughly characterized and reliable set of macrophage activation markers that would allow for improved characterization of activation patterns, and monitoring of the therapeutic efficacy of macrophage-targeted treatments.

Gene expression profiles using microarrays have been used to analyze activation of primary human monocytes and monocyte-derived macrophages (MDMs) (7, 22–32). Until very recently (33), most transcriptome-based approaches to characterize polarized macrophages contrasted two macrophage-activating conditions in each study. Using a blood sample from a single human donor, we surveyed gene expression profiles in primary macrophages activated with 33 different activating conditions. This data set served as a rich resource for identifying putative human macrophage activation markers. As a follow-up approach, integrated fluidic circuit (IFC)-based RT-PCR was used to examine a panel of transcripts to verify the reproducibility of the gene expression changes from multiple donors. This latter assay was also used to determine the expression kinetics of previously described markers of human macrophages as well as novel markers identified by the microarray-based screen.

TABLE 1 | Macrophage-activating conditions and nomenclature used in this study.

Single stimulus treatments	Previous nomenclature	Current nomenclature
1. GM-CSF (100 ng/ml)	M1	M(GM-CSF)
2. IFNβ (20 ng/ml)		M(IFNβ)
3. IFNγ (20 ng/ml)	M1, classical	M(IFNγ)
4. IL-1β (100 ng/ml)		M(IL-1β)
5. IL-4 (20 ng/ml)	M2, M2a, alternative, wound healing	M(IL-4)
6. IL-10 (50 ng/ml)	M2c	M(IL-10)
7. TGFβ (5 ng/ml)	M2c	M(TGFβ)
8. TNFα (100 ng/ml)		M(TNFα)
9. Curdlan (20 μg/ml)[a]		M(Curdlan)
10. TDB (20 μg/ml)		M(TDB)
11. Polyl:C (2 μg/ml)		M(Polyl:C)
12. LPS (10 ng/ml)	M1, classical	M(LPS10)
13. LPS (100 ng/ml)	M1, classical	M(LPS100)
14. Adenosine (100 μM)		M(Ado)
15. IgG-OVA immune complexes (IC)[a]		M(IC)
16. Dexamethasone (100 nM)	M2c	M(Dex)
Combinational treatments	**Previous nomenclature**	**Current nomenclature**
17. TDB + IFNγ		M(TDB + IFNγ)
18. TDB + IC[a]		M(TDB + IC)
19. TDB + IL-4		M(TDB + IL-4)
20. TDB + IL-10		M(TDB + IL-10)
21. LPS (10 ng/ml) + IFNγ	M1	M(LPS + IFNγ)
22. LPS (100 ng/ml) + IC[a]	M2b, regulatory	M(LPS + IC)
23. LPS (10 ng/ml) + IL-4		M(LPS + IL-4)
24. LPS (10 ng/ml) + IL-10		M(LPS + IL-10)
25. Adenosine + IFNγ		M(Ado + IFNγ)
26. Adenosine + IC[a]		M(Ado + IC)
27. Adenosine + IL-10		M(Ado + IL-10)
28. TGFβ + GM-CSF		M(TGFβ + GM-CSF)
29. TGFβ + IL-1β		M(TGFβ + IL-1β)
30. TGFβ + LPS (100 ng/ml)		M(LPS + TGFβ)
31. Dexamethasone + GM-CSF		M(Dex + GM-CSF)
32. Dexamethasone + IL-1β		M(Dex + IL-1β)
33. Dexamethasone + LPS (100 ng/ml)		M(LPS + Dex)

[a] *Treatments with chicken ovalbumin and with Curdlan likely had endotoxin contamination due to the extraction processes used to obtain these reagents (10, 11).*

Materials and Methods

Human Subjects

Human subject protocols were approved by Institutional Review Boards of the University of Iowa and the Iowa City Veterans Affairs Medical Center. Peripheral blood samples from anonymous, healthy donors were acquired through the DeGowin Blood Bank at the University of Iowa.

Integrated Fluidic Circuit-Based RT-PCR

RNA purified from MDMs using TRIzol was reverse transcribed in random hexamer-primed reactions with SuperScript III RT (Invitrogen). The cDNA was pre-amplified for 14 PCR cycles in reactions primed by a master mix of 48 TaqMan Gene Expression Assays (Applied Biosystems) using PreAmp Master Mix (Applied

Biosystems) with a modified protocol according to recommendations by Fluidigm. Following a 1:5 dilution of pre-amplified product in water, 48 samples and 48 TaqMan Gene Expression Assays were loaded onto 48.48 Dynamic Array IFC plates (Fluidigm) using the 48.48 MX IFC Controller (Fluidigm). Real-time PCR was performed using the BioMark System for Genetic Analysis (Fluidigm). Cycle threshold (Ct) values were determined using real-time PCR analysis v3.1.3 software (Fluidigm). Ct values corresponding to transcripts encoding ACTB, B2M, and TBP were used as endogenous controls. Changes in transcript expression were calculated using the ΔΔCt method and converted to $\log_2$ scale using Excel 2010. Line graphs of time course experiments were generated using Prism 6 (GraphPad). Heat maps were generated using Partek Genomic Suite software.

Cell Purification and Culture

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood by density sedimentation using Ficoll-Paque PLUS (GE Healthcare) and maintained in Petri dishes at a density of 5e7 cells/dish in 10 ml RP-10 medium [RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin, 50 μg/ml gentamicin, and 5 ng/ml M-CSF (eBioscience)]. After 10 days, non-adherent cells were removed by rinsing and the adherent MDMs were dislodged with cell scraping following incubation at 37°C for 10 min in 0.25% Trypsin/1 mM EDTA solution (Gibco). MDMs were seeded in 12-well tissue culture-treated plates (Corning) at 5e5 cells/well in 2 ml RP-10 and allowed to rest for 2 days at 37°C. Before treatment with macrophage-activating conditions, the culture medium was replaced with 1 ml fresh RP-10 per well. At 24 h post-treatment, RNA was purified from MDMs using TRIzol Reagent (Invitrogen).

Macrophage-Activating Stimuli

All stock solutions were stored at −80°C unless otherwise noted. The sources of human recombinant cytokines were as follows: IL-1β (eBioscience), IL-4 (PeproTech), IL-10 (R&D Systems), IFNβ (PeproTech), IFNγ (PeproTech), GM-CSF (eBioscience), TNFα (PeproTech), and TGFβ (R&D Systems). These cytokines were stored at concentrations recommended by the manufacturers and were subjected to no more than two freeze–thaw cycles. Dexamethasone powder (Sigma-Aldrich) was suspended in 1 part ethanol and subsequently diluted in 49 parts medium to a stock concentration of 50 μM. Phenol-extracted *Escherichia coli* 055:B5 LPS (Sigma-Aldrich) and polyinosinic:polycytidylic acid sodium salt (PolyI:C) (Sigma-Aldrich) were stored at a stock concentration of 1 mg/ml in RP-10. Adenosine (Sigma-Aldrich) was suspended in RP-10 at a stock concentration of 10 mM.

Chicken ovalbumin (MP Biomedicals) was suspended at 2 mg/ml in PBS lacking Ca^{++} or Mg^{++} (Gibco) and goat anti-chicken ovalbumin (MP Biomedicals) was suspended in water at 16 mg/ml. Immune complexes (IC) were prepared fresh for each experiment by combining ~10:1M excess of antibody to antigen and incubating with end-over-end rotation at room temperature for 30 min. Curdlan (InvivoGen) was also freshly prepared for each experiment by suspension in RP-10 at a concentration of 1 mg/ml.

Trehalose-6,6-dibehenate (TDB) (InvivoGen) was suspended at a concentration of 10 mg/ml in DMSO and heated to 60°C for 30 s. After vortexing, the TDB/DMSO solution was diluted to 1 mg/ml by the addition of PBS. This stock solution was heated to 60°C for 15 min and stored at 4°C.

Microarrays

RNA sample preparation for microarrays and the subsequent hybridization to the Illumina beadchips were performed at the University of Iowa DNA Facility. Three Human HT-12 v4 BeadChips (Illumina) were processed individually in this experiment with 1 sample from an untreated control and 11 samples from polarized macrophages loaded onto each array. Briefly, 100 ng total RNA from each of the 36 samples was amplified and converted to biotin-cRNA using the Epicenter TargetAmp-Nano Labeling Kit for Illumina Expression BeadChip (Illumina). The biotin-aRNA product was purified using the RNeasy MinElute Cleanup Kit (Qiagen) according to modifications from Epicenter. Seven hundred fifty nanograms of this product were mixed with Illumina hybridization buffer, placed onto each beadchip array, and incubated with rocking at 58°C for 17 h in an Illumina Hybridization Oven. Following hybridization, the arrays were washed, blocked, and stained with streptavidin-Cy3 using the Whole-Genome Gene Expression Direct Hybridization Assay (Illumina). Beadchip arrays were scanned with the iScan System (Illumina) and data were collected using the GenomeStudio software v2011.1 (Illumina). The expression data has been deposited in NCBI Geo repository (GSE68854).

Transcript Expression Analysis

Partek Genomic Suite v6.5 (Partek) was used to perform robust multi-array averaging and to calculate gene expression changes. A data set comprising of 1874 transcripts with changes in expression of more than fourfold relative to untreated controls was submitted to unsupervised hierarchical clustering and principal components analysis using default settings in Partek Genomic Suite software. Briefly, for unsupervised hierarchical clustering, agglomerative clustering was used to determine Euclidean dissimilarity distances using an average linkage method. For principal components analysis, a dispersion matrix based on correlations was normalized using Eigenvector scaling. Contribution of individual transcripts to each of the principal components was determined using the FactoMineR package in R. After principal component analysis was completed the contribution of each transcript to each of the components was extracted and ranked using Excel 2010.

Correlation coefficients were calculated for each pairwise combination of the 33 activated macrophage expression profiles for the 1874 regulated transcripts using the corandPvalue function of the WGCNA package in R. The data were then converted to heat maps using Excel (Microsoft).

For gene ontology (GO) analysis, the STRING database (version 9.05; string-db.org) was used to identify the 1615 protein coding RNAs in our set of 1874 regulated transcripts. Also, within the STRING database website, the GO categories enriched in the set of 1615 regulated transcripts identified as protein coding RNAs were determined.

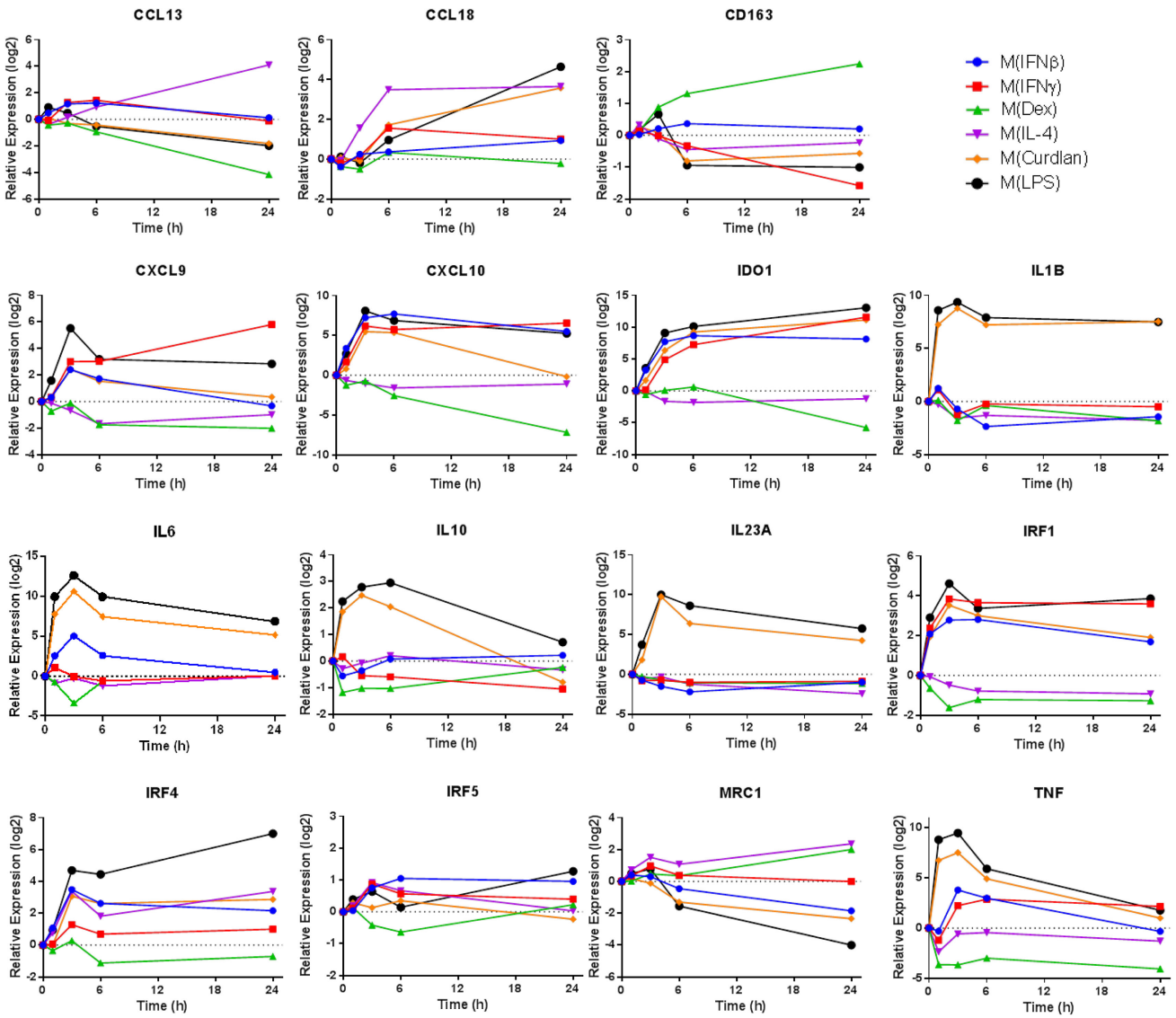

FIGURE 1 | Expression kinetics of previously proposed macrophage activation markers. At 1, 3, 6, and 24 h time points, RNA was collected from untreated MDM controls as well as M(IFNβ), M(IFNγ), M(Dex), M(IL-4), M(Curdlan), and M(LPS). Using IFC-based RT-PCR, the changes in expression for each indicated transcript relative to the untreated MDM controls was determined at each time point for the six types of activated MDMs ($N = 1$).

Results

Survey of Proposed Human Macrophage Activation Marker Expression in MDMs Responding to Six Distinct Activation Conditions

Transcripts used as markers of polarized human macrophages should change expression in response to one stimulus or a limited number of related activation stimuli. Additionally, macrophage activation markers should have sustained, rather than transient, changes in expression. In primary human macrophages responding to a variety of activation conditions, we evaluated the expression kinetics of transcripts that encode 11 proposed activation markers (4, 6, 9) over a 24-h period (**Figure 1**). Several observations from this survey were notable. First, some commonly assessed transcripts, TNF and IL-10, were rapidly induced in M(LPS) and M(Curdlan) but returned to near basal expression by the 24-h time point. Second, although many genes were similarly regulated in M(IFNβ) and M(IFNγ), the expression patterns of CD163 and CXCL9 were distinct in response to these two interferon types. Third, most markers have been noted because of their increased expression in response to macrophage activation conditions but many transcripts in this panel showed a remarkable reduction in expression. Finally, the expression level of many activation marker transcripts was either continuing to change or was sustained at high levels at the 24-h time point. Together, these observations revealed there is a need for a systematic attempt to identify reliable activation markers whose expression was either up- or down-regulated in human macrophages, and which

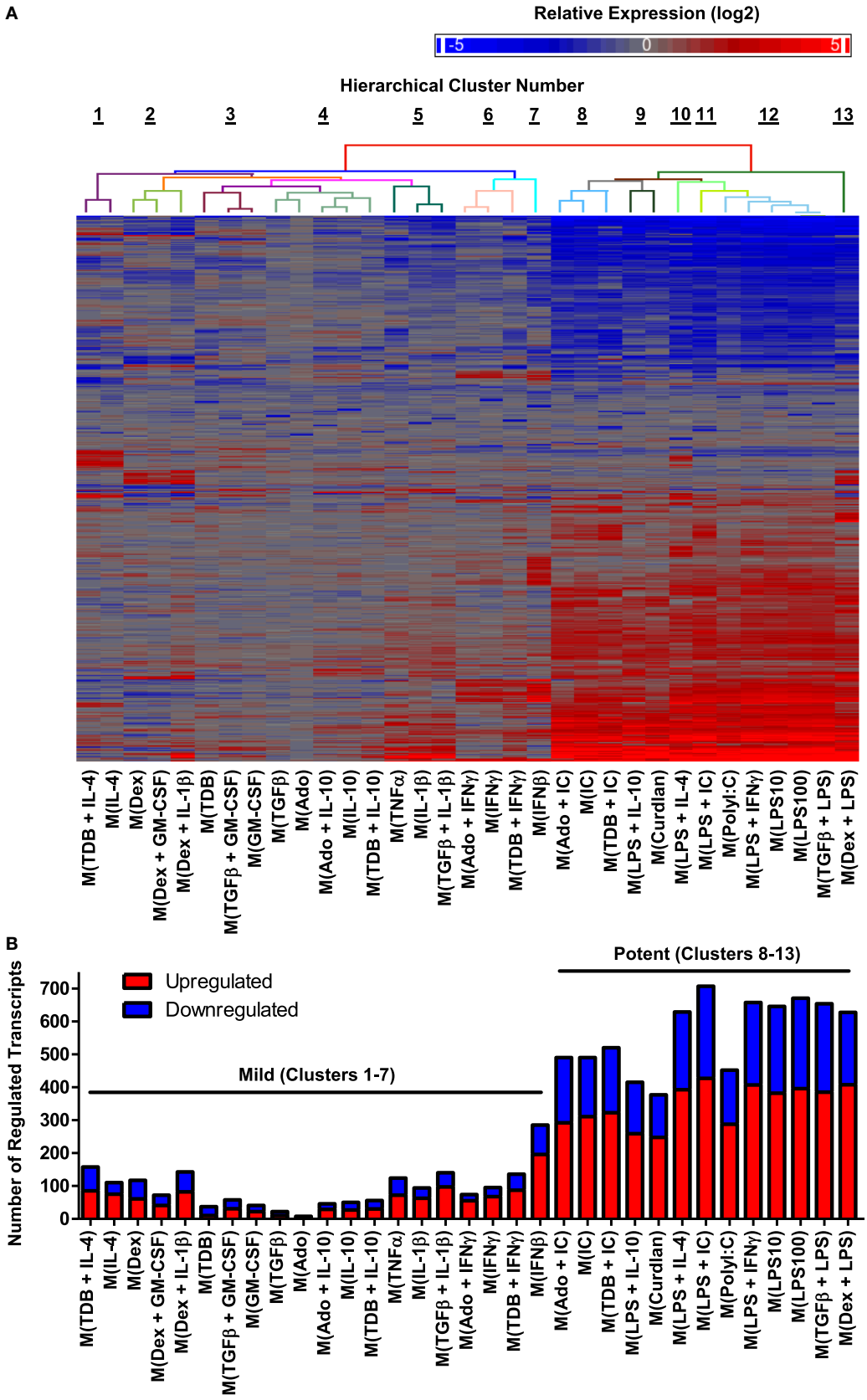

FIGURE 2 | Continued

FIGURE 2 | Continued
Hierarchical clustering of gene expression profiles from activated human MDMs separated into 2 major clusters and 13 minor clusters. Microarrays were performed using RNA collected from MDMs at 24 h post-treatment with 33 distinct activation conditions ($N = 1$). **(A)** A set of 1874 regulated transcripts defined as having >4-fold change in expression levels relative to untreated controls was compiled and displayed as a heat map ($\log_2$ scale). Gene expression profiles were sorted according to unsupervised hierarchical clustering of genes and treatments. Dissimilarity distances between gene expression profiles are displayed using a color-coded dendrogram to indicate 13 hierarchical clusters. See Section "Results" for dissimilarity distance cut-off rationale. Arranged in the same order as shown here, transcript names and quantitation of expression level changes are available in Table S1 in Supplementary Material. **(B)** Number of upregulated and down-regulated transcripts within each gene expression profile. Potent and mild macrophage activation conditions are indicated.

exhibited sustained expression level changes in response to an array of activation conditions.

Expression Profiling of a Diverse Array of Activated Human Macrophages

To screen for transcripts representing putative human macrophage activation markers, microarrays were performed using samples collected from human MDMs derived from a single donor, subjected to 33 unique activating conditions (**Table 1**) for 24 h. Sixteen of the conditions were composed of a single activating stimulus. Eight cytokines comprised the largest category of macrophage-activating stimuli used in this study and represent a spectrum of pro- and anti-inflammatory molecules that are abundantly expressed in sites where MDMs would be recruited such as infections or wounds. Pathogen-associated molecular patterns (PAMPs) recognized by C-type lectin receptors (CLRs) or toll-like receptors (TLRs) were the second largest set of macrophage-activating stimuli in this study and consisted of Curdlan (dectin-1 agonist), TDB (trehalose-6,6-dibehenate; mincle agonist), polyI:C (TLR3 agonist), or one of two concentrations of LPS (TLR4 agonist). Another set of stimuli, IgG–OVA IC and adenosine, were selected for their ability to reprogram inflammatory macrophages to become non-inflammatory (34, 35). Finally, we selected the glucocorticoid, dexamethasone, as an immunosuppressive stimulus. The remaining 17 conditions consisted of pairs of the above macrophage-activating stimuli (**Table 1**). The macrophage-activating conditions were selected with the expectation that they would lead to diverse gene expression profiles providing insights into the potential diversity of macrophage gene expression programs.

We first focused our attention on regulated transcripts that had changes in abundance of over fourfold relative to untreated controls changes. A data set of 1874 regulated transcripts that were differentially expressed in MDMs responding to one or more of the macrophage-activating conditions was compiled. Unsupervised hierarchical clustering was performed to evaluate the expression profiles of the regulated transcripts; this is summarized in a heat map that includes a dendrogram indicating relative dissimilarity distances between gene expression profiles of each polarized macrophage type (**Figure 2A**). Official gene names of the regulated transcripts and calculated expression changes are provided as supplemental material (Table S1 in Supplementary Material).

We considered whether the clustering analysis results separated the gene expression profiles corresponding to previously studied macrophage activation states as denoted in **Table 1**. Consistent with the previous reports (29, 33), gene expression profiles of M(LPS + IFNγ) (previously named "M1") macrophages were quite different from that of M(IL-4) (previously named "M2a") macrophages. By contrast, the profile of M(LPS + IC) macrophages (previously named "M2b") was very similar to the profiles of M(LPS + IFNγ), separated only by the profile of M(PolyI:C). Since M(LPS + IFNγ) and M(LPS + IC) are known to have different biological activities (6), we divided the 33 macrophage expression profiles into 13 clusters, the lowest dissimilarity distance cut-off that successfully separated these profiles (**Figure 2A**).

Microarray Results were Confirmed Using IFC Arrays

The IFC array-based real-time RT-PCR platform provided a high-throughput mechanism to accurately verify the expression of a large set of transcripts in samples from multiple human donors. We used several strategies to select a panel of transcripts with diverse expression patterns out of the 1874 regulated transcripts, which were re-assessed on multiple samples using IFC arrays. First, we included the 11 transcripts analyzed in **Figure 1**. Next, we used the STRING database (version 9.05) to identify enriched GOs for the 1615 protein coding RNAs in our set of 1874 regulated transcripts. Among the GOs categories that were enriched in our data set, we chose to focus on chemokine activity, cell surface, and cytokine activity because these GO categories were highly enriched (Table S2 in Supplementary Material). Finally, we selected transcripts that were uniquely regulated in one or two minor clusters. The final panel included a combination of transcripts that represented changes occurring in each of the 33 macrophage-activating conditions. We also mined the data set for reliable endogenous controls to include in the panel. Among the potential endogenous controls we considered, the expression levels of TBP (define) and B2M (define) transcripts appeared to be the least affected by the 33 macrophage-activating conditions (Table S1 in Supplementary Material).

The samples obtained from activated MDMs of a single donor that were analyzed by microarray were re-assessed using IFC arrays. Approximately 10 transcripts were not detected when using a pre-established Ct cut-off. Strong linear correlation for 15 representative transcripts was observed when comparing expression levels determined by microarray and by IFC arrays (Figure S1 in Supplementary Material). The remaining detectable transcripts in our panel had expression levels that also showed strong linear correlation when comparing microarray and RT-PCR results (data not shown). Overall, these results confirmed the microarray measurements using an independent approach and provide convincing evidence that IFC arrays was a dependable method for measuring transcript expression.

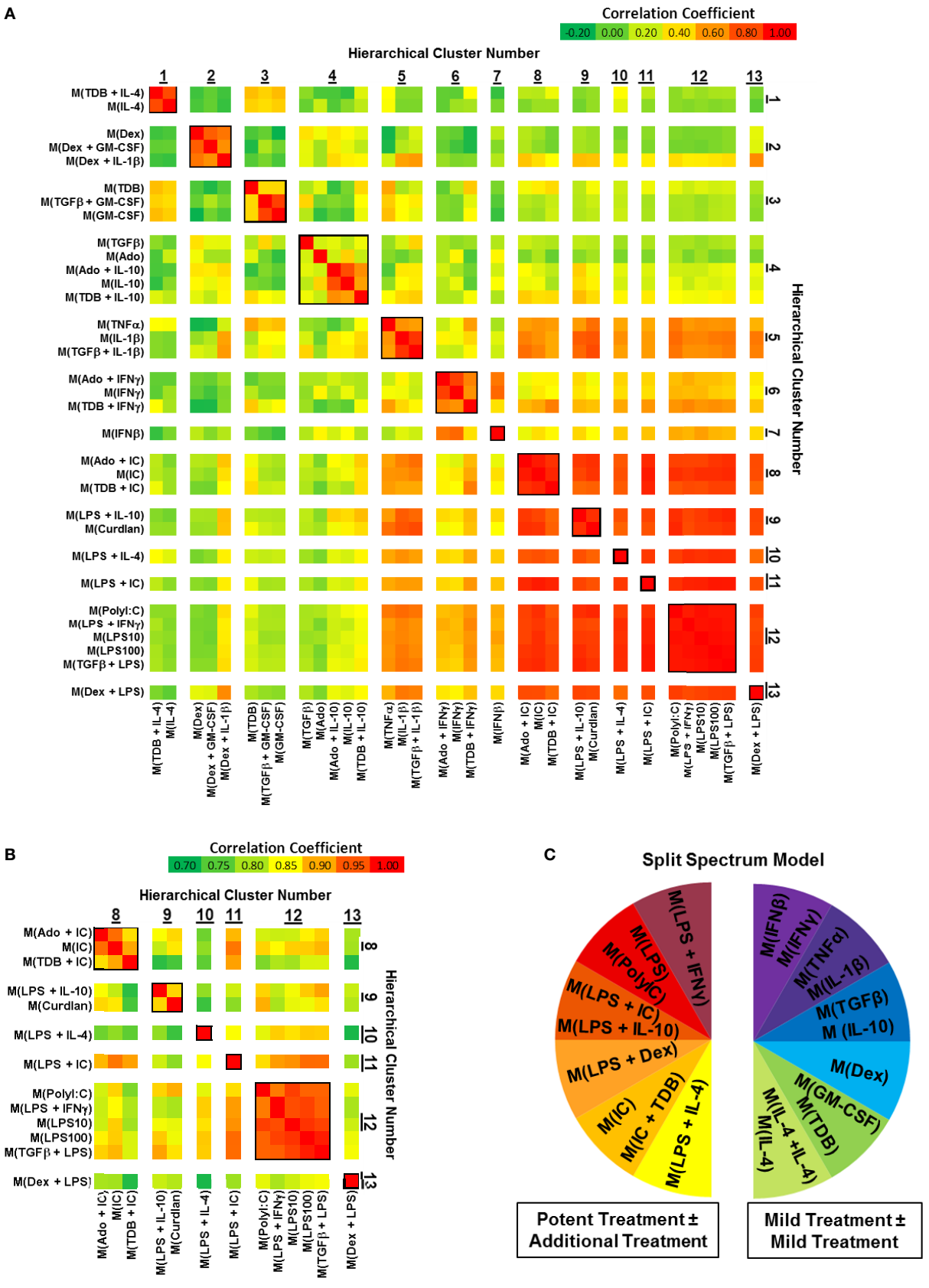

FIGURE 3 | Continued

FIGURE 3 | Continued
Comparing correlation coefficients supported the separation of gene expression profiles into two clusters, which can be modeled as a "split spectrum." Correlation coefficients were calculated for using the 1874 regulated transcript data set ($N = 1$). **(A)** Each pairwise combination of the 33 gene expression profiles are displayed as a heat map with a range of coefficients of −0.2 to 1.0. **(B)** Each pairwise combination of the 13 "potent" gene expression profiles in clusters 8–13 are displayed as a heat map with a restricted range of coefficients from 0.7 to 1.0. **(C)** A "Split Spectrum" model of macrophage activation can be used to emphasize the high degree of correlation between treatments with at least one potent macrophage-activating stimulus.

Macrophage-Activating Conditions can be Categorized as "Mild" or "Potent" Based on the Number of Transcripts regulated in Response to the Stimuli

There was a large range in the number of highly regulated transcripts in each activated macrophage expression profile (**Figure 2B**). Specifically, the 20 activated macrophage types within clusters 1–7 had relatively few regulated transcripts (93 ± 14), whereas the 13 activated macrophage types within clusters 8–13 had large numbers of regulated transcripts (564 ± 31). We propose that macrophage-activating conditions can be categorized as "mild" or "potent" based on the number of transcripts the treatment alters.

When considering the mild and potent clusters of the unsupervised hierarchical clustering, we noted that the gene expression profiles did not segregate along previously described M1–M2 divisions (**Table 1**). Polarized macrophage types, M(IFNγ) and M(LPS), which have each been considered "M1" macrophage types sorted into the mild and potent clusters, respectively. Similarly, macrophage types formerly named "M2," M(IL-4), and M(LPS + IC) were categorized as mild and potent, respectively.

We considered the possibility that the wide discrepancy in the number of transcripts regulated in MDMs responding to mild and potent activating conditions was due to suboptimal concentrations of the "mild" stimulus. To address this, MDMs were treated with each of the 11 single treatment macrophage-activating conditions that were categorized as mild at concentrations ranging from 4-fold higher to 16-fold lower those used in the microarray-based experiments. In general, modest dose responses were observed. In response to the majority of the mild stimuli tested (IFNβ, IFNγ, IL-1β, IL-4, IL-10, and TNFα), the amplitude of change in expression for any given transcript was routinely <4-fold between the lowest and highest concentrations for the activating stimulus tested (Figure S2 in Supplementary Material). This suggests that the window of activity is wide for these stimuli and further suggests that use of higher concentrations of these stimuli would be unlikely to revise their macrophage-activating categorization from "mild" to "potent."

Evaluation of Correlation Coefficients Between Activated MDM Gene Expression Profiles Supports Conclusions Drawn from Hierarchical Clustering Analyses

Correlation coefficients were determined for each pairwise combination of activated MDM gene expression profiles in the set of 1874 regulated transcripts (**Figure 3**). This analysis further substantiated the categorization of gene expression profiles into mild and potent categories as shown by unsupervised hierarchical clustering (**Figure 2**). As an example, there was a consistently higher gene expression profile correlation when the profiles of potently activated macrophages (clusters 8–13) were paired with profiles from potently activated (clusters 1–7), macrophages (**Figure 3A**). Also, we note that, when using a different color scale (**Figure 3B**), correlations between profiles in clusters 8–13 were noticeable and supported the division of the gene expression profiles of the potently activated macrophage gene expression profiles into minor clusters.

In a recent microarray-based study (33), at least 9 clusters of activated macrophages in a data set derived from human MDMs activated with 28 distinct stimuli. In agreement with the level of clustering as the previous study, we now show using unsupervised hierarchical clustering and correlation coefficient analyses that human MDMs activated with the 33 macrophage activation conditions used in this study form at least 13 clusters. Both studies support a spectrum model of macrophage activation. Because of the strong "mild" and "potent" macrophage-activating condition categories described here, we propose that macrophage activation may best be described using a "split spectrum" model (**Figure 3C**).

Verifying Transcripts that Serve as Markers for the "Potent" Macrophage Activation Conditions

The first principal component (PC1) explains (42.9%) of the variance in the data set of 1874 regulated genes while PC2 and PC3 each contributed to ~10% of the variance and the remaining principal components each accounted for <5% of the variance (**Figure 4A** and data not shown). A scatterplot of regulated gene expression profiles based on the first two principal components segregated profiles in clusters 1–7 from those in clusters 8–13 along the PC1 axis (**Figure 4B**). The expression profiles of the 50 transcripts that contributed the most to PC1 were subjected to unsupervised hierarchical clustering and displayed as a heat map (**Figure 4C**). There was an obvious distinction between gene expression responses between the profiles within the mild and potent major clusters; the transcripts robustly regulated by potent macrophage-activating conditions and relatively unaltered by mild macrophage-activating conditions underlie PC1 and account for the major source of variance for the diverse spectrum of polarized macrophage gene expression profiles in this study.

There were many transcripts in addition to the 50 noted in **Figure 4C** that contributed to PC1. Using samples collected for analysis in **Figure 1**, we monitored the change in expression of four transcripts (CCL5, IRG1, MT1G, and S100A8) that contributed to PC1 over 24 h (**Figure 4D**). CCL5 and IRG1 transcripts showed immediate increased expression levels that were sustained through the 24-h time point. By contrast, delayed increases were seen for the expression levels of MT1G and S100A8 transcripts. These four transcripts, in addition to IL1B, IL6, and IL23A that were previously seen to have sustained high expression levels in

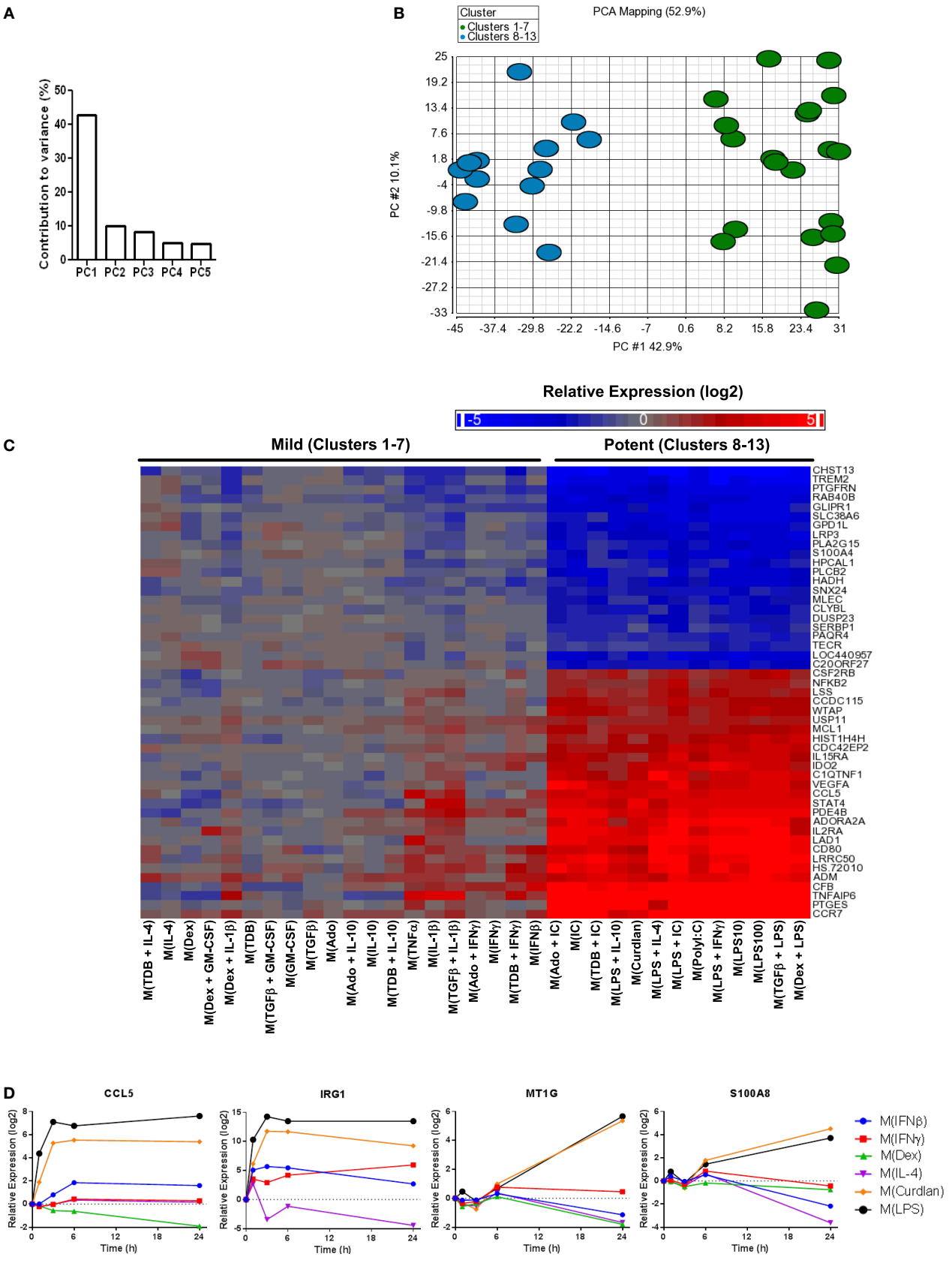

FIGURE 4 | Continued

FIGURE 4 | Continued
Transcripts universally regulated by "potent" macrophage-activating conditions in clusters 8–13 were the largest source of variance in the polarized MDM gene expression profiles. Principal components analysis was performed using the data set of 1874 regulated transcripts from the 33 gene expression profiles. **(A)** The contribution of PC1–PC5 to the variance is shown. **(B)** Scatterplot displays gene expression profiles according to PC1 and PC2 scores with color coding based on "mild" (clusters 1–7) and "potent" (clusters 8–13) categorization. **(C)** The 50 transcripts that contributed the most to PC1 were sorted according to unsupervised hierarchical clustering results. Changes in transcript expression levels relative to untreated MDMs are depicted as a heat map (log_2 scale). **(D)** Using IFC-based RT-PCR and samples from **Figure 1**, the changes in expression for each indicated transcript relative to the untreated MDM controls was determined at the 1, 3, 6, and 24 h time points for the six types of activated MDMs ($N = 1$).

M(LPS) and M(Curdlan) macrophages, suggesting that numerous transcripts that can be reliably used as markers of "potent" activation conditions.

Evaluating the Use of Chemokine Transcripts as Macrophage Activation Markers

Chemokines not only play an important functional role in macrophage activity but also include some of the earliest proposed markers of macrophage polarization (2, 5). We generated a heat map of transcript expression changes for chemokines from the C–C and C–X–C subfamilies from the 1874 transcripts (**Figure 5A**). Since IL-4 treated macrophages have been well characterized, the chemokines were sorted according to their average expression in the two activated macrophage types that form cluster 1, M(IL-4) and M(TDB + IL-4). Among the remaining 12 clusters, the chemokine expression profiles from macrophages in cluster 3, comprised of M(TDB), M(TGFβ + GM-CSF), and M(GM-CSF) macrophages, appeared to have the most similar trend in chemokine expression. The overall chemokine expression patterns from all other profiles shared little resemblance to those in cluster 1.

Transcripts for two chemokines, CCL13 and CCL22, accumulated in macrophages treated with IL-4 for 24 h (**Figure 5A**). Interestingly, the upregulation of these chemokines in response to IL-4 was delayed relative to other treatments that induced transient upregulation: interferons for CCL13 (**Figure 1**) and PAMPs for CCL22 (**Figure 5B**). These observations suggests that CCL13 and CCL22 can be used as specific markers for M(IL-4) as long as enough time has elapsed since the activation occurred.

We noted that nearly all chemokines had reduced expression in M(Dex) macrophages according to the microarray results (**Figure 5A**). This observation was confirmed when monitoring the kinetics of expression for five chemokine transcripts described above (CCL5, CCL13, CCL18, CXCL9, and CXCL10) (**Figures 1** and **4D**) and in four additional chemokines (CCL2, CCL3, CCL22, and CXCL5) (**Figure 5B**). The general trend of repressing chemokine production in M(Dex) macrophages may hint at a mechanism by which dexamethasone acts as an immunosuppressive molecule.

Donor-to-Donor Variability in Gene Expression Regulation was Minimal in Most Circumstances but was Occasionally seen in some Minor Clusters

A caveat to the results described until this point is that they were based on MDMs derived from two human donors: one donor for monitoring transcript expression kinetics and one donor for transcriptional profiling. Since donor-to-donor variability among human MDM responses was a concern, the expression profiles for many transcripts was determined in samples derived from the microarray experiment and from two additional donors whose MDMs were treated with all 33 macrophage activation conditions (**Figure 6**).

The strong correlation between the microarray results and the IFC PCR results for the first donor was discussed above (Figure S1 in Supplementary Material). Importantly, the last two rows for each transcript, which show the results for the two additional donors, indicated that the MDM responses were, in general, similar to those of the first donor (**Figure 6**). There were a few transcripts (CCL22, CXCL10, IL10, ITGB7, and TGM2) that had strong opposing changes in expression from one donor to the next (**Figure 6**). It is noteworthy that in these instances, the difference in expression was restricted to a limited number of clusters. For example, CCL22 expression regulation tended to be similar in response to all 33 macrophages activation conditions for all 3 donors; the notable exception was seen in the 5 macrophages activation conditions within cluster 12 for the second donor (**Figure 6**). This result is unlikely due to the polyIC and LPS treatments being suboptimal in the experiment involving MDMs from donor 2 since other transcripts, such as CCL5, were regulated similarly in all three donors for the macrophage-activating conditions that make up cluster 12.

A recent mass cytometry-based study produced a high-dimension data set from a panel of 38 antibodies to effectively identify signature expression patterns of myeloid cell populations in mice from a number of tissues (36). Since the dimensionality of data sets produced by mass cytometry and IFC PCR are similar, we tested whether the 13 clusters originally defined by unsupervised hierarchical clustering of the 1874 regulated transcripts (**Figure 2**) could be effectively identified using IFC PCR results (**Figure 7A**). The majority of the 13 clusters remained clusters for each of the three donors (**Figure 7B**). Even the "clusters" composed of a single type of activated macrophage type [i.e., M(IFNβ)] maintained their distinctness relative to the other activated macrophage types. We conclude that gene expression platforms such as IFC PCR monitor a large enough set of macrophage activation marker transcripts to identify an overall macrophage population's type/cluster while still allowing for detection of subtle donor-to-donor differences.

Putative Activation Markers were Identified for Specific Clusters of Polarized Human Macrophages

Macrophage activation markers would ideally have large expression changes in a single cluster or polarized macrophage type. We therefore queried the gene expression profiles in the current

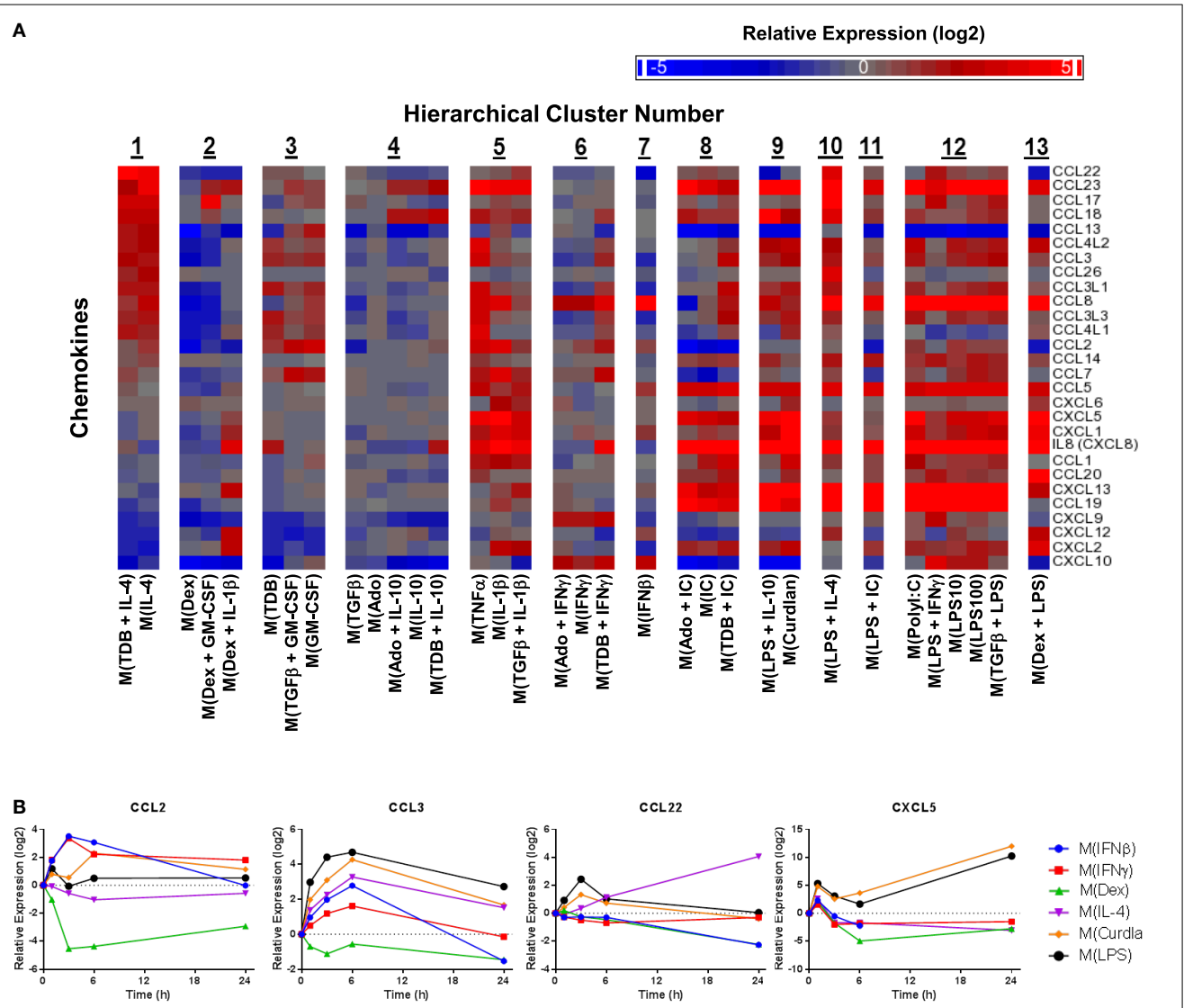

FIGURE 5 | Evaluation of chemokines as MDM activation markers. **(A)** All C–C and C–X–C chemokines were selected from the set of 1874 regulated transcripts and sorted according to average expression level changes in response to the two macrophage-activation treatment conditions within cluster 1. **(B)** Using IFC-based RT-PCR and samples from **Figure 1**, the changes in expression for each indicated transcript relative to the untreated MDM controls was determined at the 1, 3, 6, and 24 h time points for the six types of activated MDMs ($N = 1$).

study to identify activation markers specific to each of the 13 clusters formed by the unsupervised hierarchical clustering analysis. Many putative activation markers were identified in macrophages activated with IL-4, dexamethasone, or IFNβ (**Figures 8**–**10**).

IL-4 was used as an activation condition for gene expression profiles in clusters 1 and 10. We identified transcripts that were strongly upregulated only within cluster 1, within both cluster 1 and cluster 10, or only within cluster 10. Examples of transcripts that fit these gene expression profiles were readily detected within our data set (**Figure 8A**). Analysis of the kinetics of expression for three of the transcripts identified by this screening approach showed that while ALOX15 and CD1B each appear to be good markers for M(IL-4), although the increase in CD1B was delayed until the 24 h time point, FABP4 was not robustly induce in M(IL-4) but could still be a valuable marker as this transcript was potently down-regulated in response to several macrophage-activating conditions (**Figure 8B**). This latter observation was consistent with the microarray data (**Figure 8A**).

Given the relative ease of finding IL-4-associated activation markers in our data set, we switched our attention to identifying additional activation markers. Dexamethasone-associated activation markers were identified that were specifically upregulated in macrophage-activating conditions from only within cluster 2, within both clusters 2 and 13, and only within cluster 13 (**Figure 9A**). The expression kinetics was determined for three of the transcripts identified by the microarray screen as dexamethasone responsive (**Figure 9B**). Of these, ALOX15B and MFGE8 appear to be a markers for M(Dex) at early and late time points, respectively.

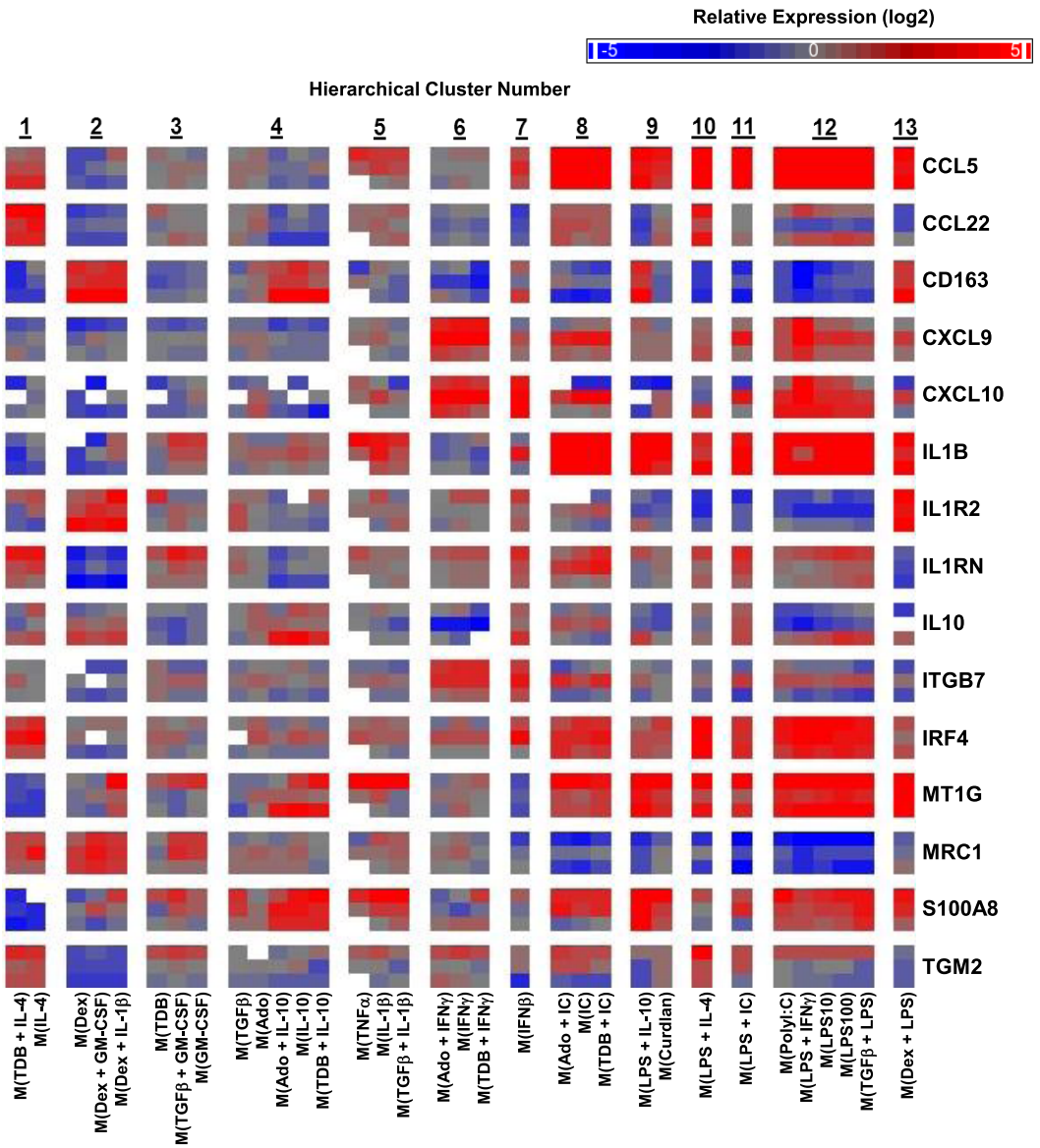

FIGURE 6 | Variability in donor-to-donor MDM gene expression responses was often limited to specific clusters. IFC-based RT-PCR was used to determine the expression of 48 transcripts (45 putative macrophage activation markers and 3 endogenous controls) in MDMs at 24 h post-treatment with 33 distinct activation conditions (columns) ($N = 3$). Shown here are the results for 15 of the activation marker transcripts. The RNA collected from the first donor (first row for each indicated transcript) had been used in the microarray studies and the RNA from two additional donors (second and third row for each indicated transcript) was collected in independent experiments. Blank areas within clusters represent samples did not meet the Ct cut-off of 25 or, in the case of the third M(TNFα) sample, did not load properly into the IFC device.

Next, potential activation markers or IFNβ-treated macrophages were identified within cluster 7 (**Figure 10A**). Further analysis showed that AXL, IFIT, and ZBP1 were all induced rapidly in M(IFNβ) and with delayed kinetics in M(LPS) (**Figure 10B**). This observation may be explained by indirect induction of these genes by LPS-induced IFNβ production.

Discussion

Characterization of TAMs has shifted from quantifying macrophage density in and around tumors to evaluating markers of activation (15, 37). It is important to note that macrophage activation markers have been used to categorize macrophage activation, typically using the M1–M2 nomenclature, yet the regulation patterns of these markers in macrophages responding to a wide variety of activation conditions are not well understood. Using a combined microarray- and IFC array-based approach in this study, previously proposed markers of macrophage activation were better characterized and novel markers of macrophage activation were identified.

In the earliest report using M1–M2 nomenclature, the authors stated that "M-1 and M-2, while useful for conceptualizing

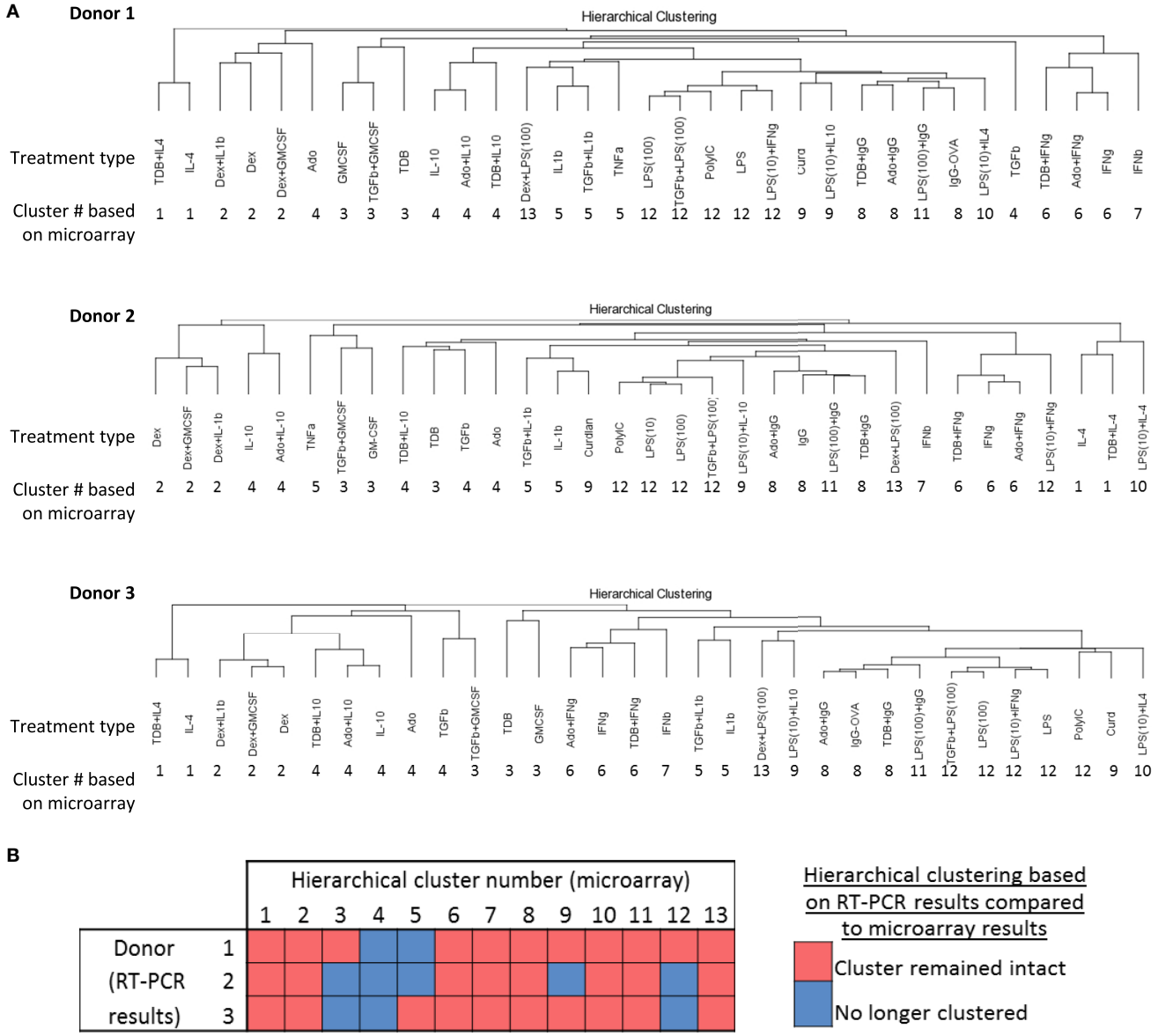

FIGURE 7 | Comparing unsupervised hierarchical clustering of 33 activated macrophage types based on 1874 regulated transcripts against hierarchical clustering based on a 45-transcript subset of putative activation markers. IFC PCR was used to determine the expression of 48 transcripts in MDMs at 24 h post-treatment with 33 distinct activation conditions (columns) ($N = 3$). The RNA collected from the first donor had been used in the microarray studies and the RNA from two additional donors was collected in independent experiments. Data points were omitted when the ΔCt value was unreliable as defined by either the macrophage activation marker or the endogenous control not meeting the Ct cut-off of 25. **(A)** Unsupervised hierarchical clustering was performed using calculated ΔΔCt values derived from IFC PCR. Dissimilarity distances between gene expression profiles are displayed as dendrograms for each donor. For comparison purposes, the hierarchical cluster number is displayed below each macrophage-activating treatment type. **(B)** A summary is shown for comparisons between microarray-derived clusters from donor 1 and IFC PCR-derived clusters from donors 1, 2, and 3.

immune responses, certainly could be an oversimplification" and that "there may be a continuum of phenotypes between M-1 and M-2 macrophages" (3). A recently proposed framework argued against using the M1–M2 nomenclature yet upheld the linear model concept that suggested M(IFNγ) and M(IL-4) to represent the polar extremes (9). However, both the results of the current study and those reported by Xue et al. (33) support a spectrum model of macrophage activation rather than a linear model.

Unsupervised hierarchical clustering, correlation coefficient analysis, and principal components analysis of the regulated transcripts each support the concept that macrophage polarized states in this study can be sorted into two major clusters. We designated these clusters "mild" and "potent" to convey the number transcripts altered in response to each specific macrophage-activating condition. It is important to note that, although we have evaluated more macrophage activation conditions in a macrophage

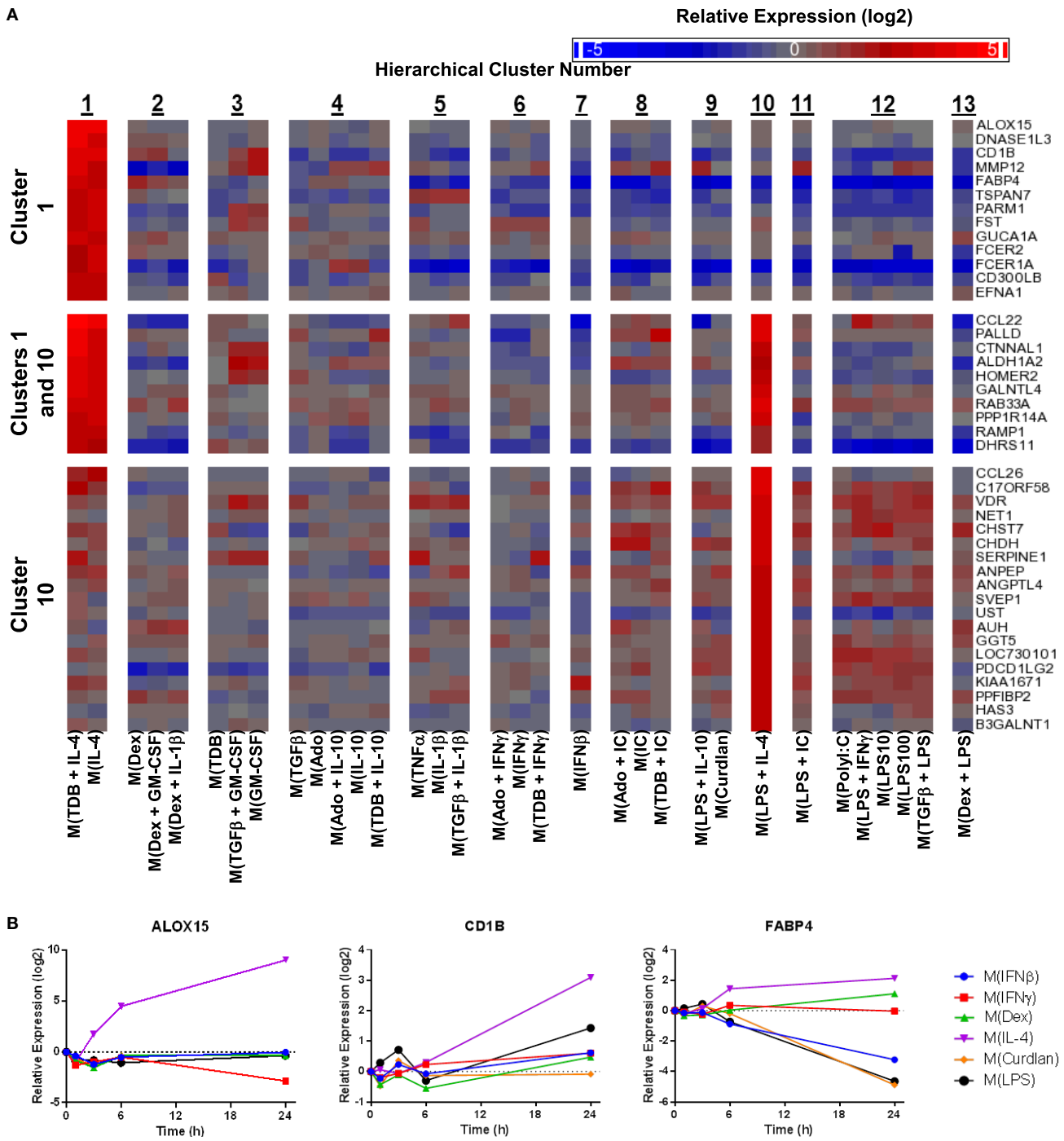

FIGURE 8 | Evaluation of activation markers in MDMs responding to treatments with IL-4. **(A)** Putative macrophage activation markers were screened for within the microarray data that met two criteria: (i) a >4-fold expression level change in response to activation conditions that included IL-4 (samples within cluster 1 and/or cluster 10) relative to untreated MDMs and (ii) a >2-fold expression level change relative to the activating conditions that did not include IL-4. **(A)** Changes in select putative activation markers as determined by microarray analysis are shown as a heat map ($\log_2$ scale) ($N = 1$) **(B)** Using IFC-based RT-PCR and samples from **Figure 1**, the changes in expression for each indicated transcript relative to the untreated MDM controls was determined at the 1, 3, 6, and 24 h time points for the six types of activated MDMs ($N = 1$).

activation study that has previously been published, there could be activation conditions that will have an intermediate number of regulated transcripts making our split spectrum model potentially incorrect. Indeed, Xue et al. (33) studied macrophage responses to 28 activation conditions and we found that the free fatty acid conditions from their study may represent an "intermediate"

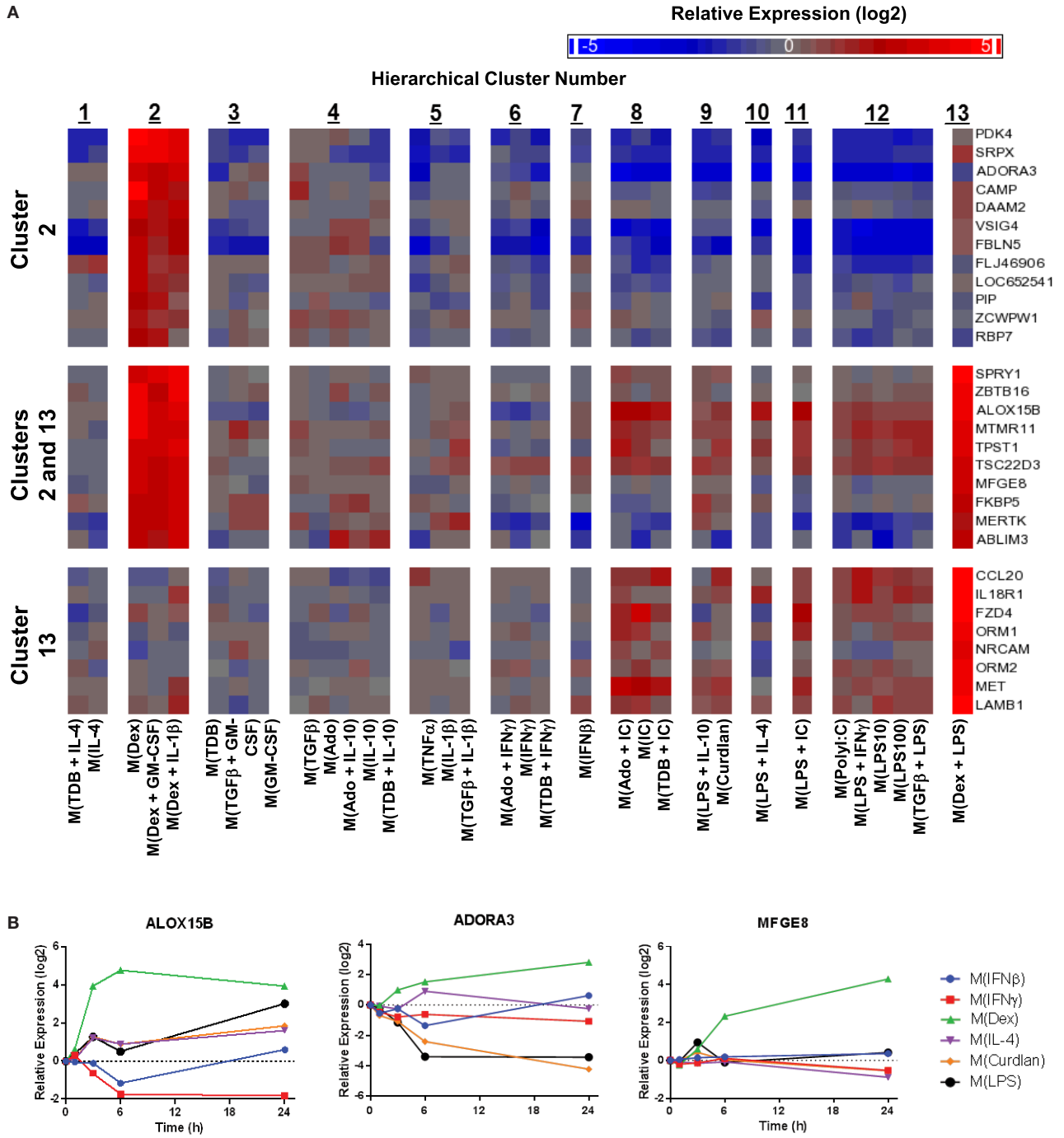

FIGURE 9 | Evaluation of activation markers in MDMs responding to treatments with dexamethasone. **(A)** Putative macrophage activation markers were screened for within the microarray data that met similar criteria as described in **Figure 7A** with a focus on transcripts that changed in response dexamethasone treatment (samples within clusters 2 and/or 13). **(B)** Using IFC-based RT-PCR and samples from **Figure 1**, the changes in expression for each indicated transcript relative to the untreated MDM controls was determined at the 1, 3, 6, and 24 h time points for the six types of activated MDMs ($N = 1$).

cluster (analysis not shown). While our "split spectrum" model may not represent the entirety of the spectrum, it raises the idea that strength of macrophage activation may be worth considering in future attempts to accurately describe macrophage activation/polarization.

In the analysis of the principal components, special attention was warranted for PC1 because it accounted for four times more of the variance than any other principal component. The single treatment macrophage-activating conditions that contributed to PC1 were immune complexes, Curdlan, polyIC, and LPS.

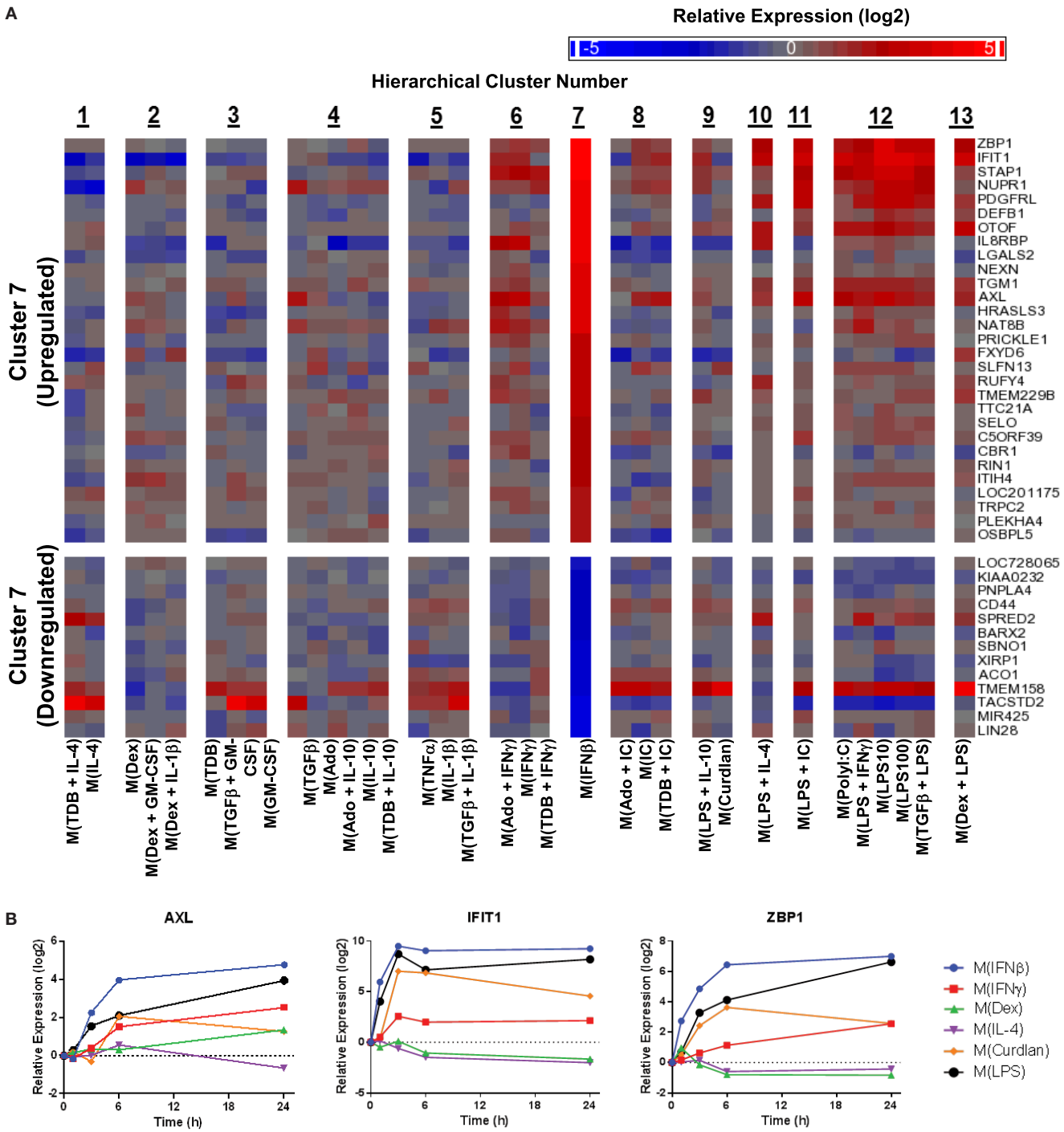

FIGURE 10 | Evaluation of activation markers in MDMs responding to treatment with IFNβ. **(A)** Putative macrophage activation markers were screened for within the microarray data that met similar criteria as described in **Figure 7A** with a focus on transcripts that changed in response IFNβ treatment (cluster 7). **(B)** Using IFC-based RT-PCR and samples from **Figure 1**, the changes in expression for each indicated transcript relative to the untreated MDM controls was determined at the 1, 3, 6, and 24 h time points for the six types of activated MDMs ($N = 1$).

All combinational treatments that contributed to PC1 contained one or two of these potent stimuli. Treatment of macrophages with immune complexes and Curdlan initially signal through Fcγ receptor/Syk/Card9 pathways while treatment with polyIC and LPS signal through TRIF and/or MyD88 pathways. Despite these initial differences, there is substantial overlap triggered by the potent stimuli further downstream pathway signaling. For example, activation of pathways such as NF-κB and MAPK may

directly and indirectly account for the regulated expression of many transcripts that contributed to PC1. Importantly, as noted in **Table 1**, chicken ovalbumin and Curdlan are often contaminated with substantial levels of endotoxin, so the "potent" activation conditions may be mostly or in part a consequence of TLR-initiated signaling (10, 11). Future studies will assess the extent that TLR signaling may have contributed to the alterations in the M(Curdlan) and M(IC) macrophage gene expression profiles.

There was substantial evidence, both gene expression and functional, that the mild and potent polarized macrophage types of our data set should be divided into smaller clusters. To define these clusters, we chose to separate our gene expression profiles based on known differences that occur in response macrophage-activating conditions rather than using a statistically based dissimilarity cut-off in the unsupervised hierarchical clustering. Specifically, we noted that M(LPS + IFNγ) and M(LPS + IC) were situated close to each other according to unsupervised hierarchical clustering analysis (**Figure 2**). Important functional differences in macrophages treated with these two distinct activating conditions such as cytokine production (IL-12 vs. IL-10) and ability to skew $CD4^+$ T cell responses (Th1 vs. Th2) (35, 38–40) supported the segregation of these gene expression profiles into separate clusters. Therefore, the dissimilarity distance between these two gene expression profiles served as our cut-off to rationally sort the 33 gene expression profiles into 13 clusters.

It is notable that if the gene expression profiles had been segregated based on dissimilarity distances into 14 clusters instead of 13, the 5 gene expression profiles currently grouped within "cluster 4" would have been split into 2 clusters. Furthermore, correlation coefficients within cluster 4 were markedly higher when comparing gene expression profiles from MDMs activated with conditions that included IL-10 (**Figure 3**). Finally, hierarchical clustering based on IFC PCR results (**Figure 7**) failed to retain the integrity of cluster 4 in any of the three donors. These observations suggest that subdividing the 33 gene expression profiles into more than 13 clusters may have been warranted starting with subdividing cluster 4. Future functional studies will be useful for supporting or modifying our current classification of 13 clusters for these 33 macrophage-activating conditions.

In order for macrophage activation markers to be useful, it is critical to know whether each marker is regulated by a wide variety or a limited number of stimuli. In our initial time course analysis survey of previously proposed macrophage activation markers, few of the 11 transcripts were found to be highly specific for a specific type of activated macrophage. Therefore, microarrays were performed and then surveyed to identify novel macrophage activation markers. This approach proved to be useful for identifying markers differentially expressed by activated macrophages in all the potent conditions used in this study (**Figure 4**) and in many of the minor clusters (**Figures 7–9**).

Our approach of screening for macrophage activation markers by surveying microarray results of a single donor's macrophage responses to 33 different activation conditions and following up with IFC arrays proved effective. Also, use of unbiased, bottom-up analyses of the microarray results argue against previously proposed top-down linear frameworks describing macrophage activation states, such as the M1–M2 system (3, 9). We note that our results are in line with the spectrum model proposed by Xue et al. from their microarray data set (33). There are likely to be more clusters of activated macrophages than the 13 described here and the 9 described by Xue et al. (33). Taken together, we conclude that measuring the expression changes in a panel of well-characterized markers would provide a useful tool to accurately differentiate various activation states associated with functional activity of TAMs or other macrophage populations.

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Supplementary Material

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2015.00253

Table S1 | Gene expression profiles in the 33 activated macrophage types for the 1874 regulated transcripts.

Table S2 | GO enrichment for 1615 gene names recognized by STRING v. 9.05.

Figure S1 | IFC PCR-calculated transcript expression changes correlated well with results from the microarrays. Scatterplots of gene expression level changes of the indicated transcripts as determined by microarray and by Fluidigm IFC-based RT-PCR. RNA samples collected at 24 h post-treatment from activated MDMs of a single donor were used as template for both assays.

Figure S2 | Dose-dependent changes in MDM transcript expression levels were minimal across a broad range of concentrations for most of the mild, single stimulus treatments. IFC-based RT-PCR was used to monitor the expression of 48 transcripts in MDMs from a single donor treated with four different concentrations of 11 indicated mild treatment stimuli. The concentrations tested were 4×, 1×, 1/4×, and 1/16× relative to the concentration described for each stimulus in **Table 1**. For each treatment, transcripts that had at least a fourfold change in expression (>2 or <−2 on $\log_2$ scale) in any of the four tested concentrations were selected for display.

References

1. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* (1992) **176**:287–92. doi:10.1084/jem.176.1.287
2. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear

phagocytes. *Trends Immunol* (2002) **23**:549–55. doi:10.1016/S1471-4906(02)02302-5
3. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* (2000) **164**:6166–73. doi:10.4049/jimmunol.164.12.6166
4. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* (2008) **8**:958–69. doi:10.1038/nri2448
5. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* (2004) **25**:677–86. doi:10.1016/j.it.2004.09.015
6. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* (2008) **13**:453–61. doi:10.2741/2692
7. Gleissner CA, Shaked I, Little KM, Ley K. CXC chemokine ligand 4 induces a unique transcriptome in monocyte-derived macrophages. *J Immunol* (2010) **184**:4810–8. doi:10.4049/jimmunol.0901368
8. Zizzo G, Cohen PL. IL-17 stimulates differentiation of human anti-inflammatory macrophages and phagocytosis of apoptotic neutrophils in response to IL-10 and glucocorticoids. *J Immunol* (2013) **190**:5237–46. doi:10.4049/jimmunol.1203017
9. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* (2014) **41**:14–20. doi:10.1016/j.immuni.2014.06.008
10. Sonck E, Devriendt B, Goddeeris B, Cox E. Varying effects of different beta-glucans on the maturation of porcine monocyte-derived dendritic cells. *Clin Vaccine Immunol* (2011) **18**:1441–6. doi:10.1128/CVI.00080-11
11. Watanabe J, Miyazaki Y, Zimmerman GA, Albertine KH, McIntyre TM. Endotoxin contamination of ovalbumin suppresses murine immunologic responses and development of airway hyper-reactivity. *J Biol Chem* (2003) **278**:42361–8. doi:10.1074/jbc.M307752200
12. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol* (2008) **66**:1–9. doi:10.1016/j.critrevonc.2007.07.004
13. Coussens LM, Werb Z. Inflammation and cancer. *Nature* (2002) **420**:860–7. doi:10.1038/nature01322
14. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* (2004) **4**:71–8. doi:10.1038/nrc1256
15. Van Overmeire E, Laoui D, Keirsse J, Van Ginderachter JA, Sarukhan A. Mechanisms driving macrophage diversity and specialization in distinct tumor microenvironments and parallelisms with other tissues. *Front Immunol* (2014) **5**:127. doi:10.3389/fimmu.2014.00127
16. Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol* (2005) **175**:342–9. doi:10.4049/jimmunol.175.1.342
17. Stout RD, Suttles J. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. *J Leukoc Biol* (2004) **76**:509–13. doi:10.1189/jlb.0504272
18. Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, et al. Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell* (2013) **24**:589–602. doi:10.1016/j.ccr.2013.09.014
19. Pallasch CP, Leskov I, Braun CJ, Vorholt D, Drake A, Soto-Feliciano YM, et al. Sensitizing protective tumor microenvironments to antibody-mediated therapy. *Cell* (2014) **156**:590–602. doi:10.1016/j.cell.2013.12.041
20. Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med* (2013) **19**:1264–72. doi:10.1038/nm.3337
21. Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* (2014) **25**:846–59. doi:10.1016/j.ccr.2014.05.016
22. Bosco MC, Puppo M, Santangelo C, Anfosso L, Pfeffer U, Fardin P, et al. Hypoxia modifies the transcriptome of primary human monocytes: modulation of novel immune-related genes and identification of CC-chemokine ligand 20 as a new hypoxia-inducible gene. *J Immunol* (2006) **177**:1941–55. doi:10.4049/jimmunol.177.3.1941
23. Bostrom P, Magnusson B, Svensson PA, Wiklund O, Boren J, Carlsson LM, et al. Hypoxia converts human macrophages into triglyceride-loaded foam cells. *Arterioscler Thromb Vasc Biol* (2006) **26**:1871–6. doi:10.1161/01.ATV.0000229665.78997.0b
24. Chang YC, Chen TC, Lee CT, Yang CY, Wang HW, Wang CC, et al. Epigenetic control of MHC class II expression in tumor-associated macrophages by decoy receptor 3. *Blood* (2008) **111**:5054–63. doi:10.1182/blood-2007-12-130609
25. Hu X, Park-Min KH, Ho HH, Ivashkiv LB. IFN-gamma-primed macrophages exhibit increased CCR2-dependent migration and altered IFN-gamma responses mediated by Stat1. *J Immunol* (2005) **175**:3637–47. doi:10.4049/jimmunol.175.6.3637
26. Irvine KM, Andrews MR, Fernandez-Rojo MA, Schroder K, Burns CJ, Su S, et al. Colony-stimulating factor-1 (CSF-1) delivers a proatherogenic signal to human macrophages. *J Leukoc Biol* (2009) **85**:278–88. doi:10.1189/jlb.0808497
27. Jura J, Wegrzyn P, Korostynski M, Guzik K, Oczko-Wojciechowska M, Jarzab M, et al. Identification of interleukin-1 and interleukin-6-responsive genes in human monocyte-derived macrophages using microarrays. *Biochim Biophys Acta* (2008) **1779**:383–9. doi:10.1016/j.bbagrm.2008.04.006
28. Locati M, Deuschle U, Massardi ML, Martinez FO, Sironi M, Sozzani S, et al. Analysis of the gene expression profile activated by the CC chemokine ligand 5/RANTES and by lipopolysaccharide in human monocytes. *J Immunol* (2002) **168**:3557–62. doi:10.4049/jimmunol.168.7.3557
29. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* (2006) **177**:7303–11. doi:10.4049/jimmunol.177.10.7303
30. Scotton CJ, Martinez FO, Smelt MJ, Sironi M, Locati M, Mantovani A, et al. Transcriptional profiling reveals complex regulation of the monocyte IL-1 beta system by IL-13. *J Immunol* (2005) **174**:834–45. doi:10.4049/jimmunol.174.2.834
31. Sironi M, Martinez FO, D'Ambrosio D, Gattorno M, Polentarutti N, Locati M, et al. Differential regulation of chemokine production by Fcgamma receptor engagement in human monocytes: association of CCL1 with a distinct form of M2 monocyte activation (M2b, Type 2). *J Leukoc Biol* (2006) **80**:342–9. doi:10.1189/jlb.1005586
32. Tassiulas I, Hu X, Ho H, Kashyap Y, Paik P, Hu Y, et al. Amplification of IFN-alpha-induced STAT1 activation and inflammatory function by Syk and ITAM-containing adaptors. *Nat Immunol* (2004) **5**:1181–9. doi:10.1038/ni1126
33. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* (2014) **40**:274–88. doi:10.1016/j.immuni.2014.01.006
34. Cohen HB, Briggs KT, Marino JP, Ravid K, Robson SC, Mosser DM. TLR stimulation initiates a CD39-based autoregulatory mechanism that limits macrophage inflammatory responses. *Blood* (2013) **122**:1935–45. doi:10.1182/blood-2013-04-496216
35. Sutterwala FS, Noel GJ, Salgame P, Mosser DM. Reversal of proinflammatory responses by ligating the macrophage Fcgamma receptor type I. *J Exp Med* (1998) **188**:217–22. doi:10.1084/jem.188.1.217
36. Becher B, Schlitzer A, Chen J, Mair F, Sumatoh HR, Teng KW, et al. High-dimensional analysis of the murine myeloid cell system. *Nat Immunol* (2014) **15**:1181–9. doi:10.1038/ni.3006
37. Quatromoni JG, Eruslanov E. Tumor-associated macrophages: function, phenotype, and link to prognosis in human lung cancer. *Am J Transl Res* (2012) **4**:376–89.
38. Anderson CF, Mosser DM. A novel phenotype for an activated macrophage: the type 2 activated macrophage. *J Leukoc Biol* (2002) **72**:101–6.
39. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. *J Leukoc Biol* (2006) **80**:1298–307. doi:10.1189/jlb.0406249
40. Gerber JS, Mosser DM. Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. *J Immunol* (2001) **166**:6861–8. doi:10.4049/jimmunol.166.11.6861

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