



# Function of Macrophages in Disease: Current Understanding on Molecular Mechanisms

Chunye Zhang<sup>1</sup>, Ming Yang<sup>2\*</sup> and Aaron C. Ericsson<sup>1,3,4\*</sup>

<sup>1</sup> Department of Veterinary Pathobiology, University of Missouri, Columbia, MO, United States, <sup>2</sup> Department of Surgery, University of Missouri, Columbia, MO, United States, <sup>3</sup> Department of Veterinary Pathobiology, University of Missouri Metagenomics Center, University of Missouri, Columbia, MO, United States, <sup>4</sup> Department of Veterinary Pathobiology, University of Missouri Mutant Mouse Resource and Research Center, Columbia, MO, United States

Tissue-resident macrophages (TRMs) are heterogeneous populations originating either from monocytes or embryonic progenitors, and distribute in lymphoid and non-lymphoid tissues. TRMs play diverse roles in many physiological processes, including metabolic function, clearance of cellular debris, and tissue remodeling and defense. Macrophages can be polarized to different functional phenotypes depending on their origin and tissue microenvironment. Specific macrophage subpopulations are associated with disease progression. In studies of fate-mapping and single-cell RNA sequencing methodologies, several critical molecules have been identified to induce the change of macrophage function. These molecules are potential markers for diagnosis and selective targets for novel macrophage-mediated treatment. In this review, we discuss some of the recent findings regarding less-known molecules and new functions of well-known molecules. Understanding the mechanisms of these molecules in macrophages has the potential to yield new macrophage-mediated treatments or diagnostic approaches to disease.

**Keywords:** macrophage, phenotype, molecules, diagnostic marker, therapy

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### \*Correspondence:

Aaron C. Ericsson  
ericssona@missouri.edu  
Ming Yang  
yangmin@health.missouri.edu

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## INTRODUCTION

Macrophages are an essential component of the innate immune system, with a wide distribution in lymphoid and non-lymphoid tissues throughout the body. Macrophages were initially known to arise from circulating blood monocytes that continuously migrate to different tissues and differentiate into macrophages (1). Although the origin of adult tissue-resident macrophages (TRMs) is still not totally understood, it is now recognized that TRMs are derived from diverse progenitors, including embryonic origin and monocyte progenitors. Some fate-mapping studies have demonstrated that major TRMs in mice, such as liver resident Kupffer cells (KCs) and lung alveolar, splenic, and peritoneal macrophages, are developed before birth and can maintain themselves independent of circulating blood monocytes (2, 3). Further study also demonstrated that those TRMs such as KCs in mouse fetal liver originate from yolk sac erythro-myeloid progenitors (EMPs), which are distinct from adult hematopoietic stem cells (HSCs) (4). In contrast, other studies showed that some TRMs such as adult cardiac and skeletal muscle macrophages are derived both from yolk-sac and fetal monocyte progenitors (5, 6), and embryonically developed TRMs can be replaced by blood monocytes.

Macrophages have pivotal functions in homeostasis and many physiological processes beyond innate immunity, including metabolic function (7), clearance of cellular debris (8), tissue repair

and remodeling (9). In pathogenic conditions, TRMs can be replaced and joined by recruited monocyte-derived macrophages to orchestrate an immune response (10). Current studies using single-cell RNA-sequencing (scRNA-seq) have revealed the presence of multiple macrophage subsets with distinct functions in various tissues (11). For example, Remmerie et al. (12) reported that in fatty liver, hepatic resident KCs were destroyed and replaced by bone marrow-derived macrophages, and those recruited macrophages comprise two subsets resembling either KCs or lipid-associated macrophages expressing Osteopontin. Moreover, macrophage function and phenotype are impacted by various factors in physiological and pathological conditions, such as diet (13) and cytokines (14). The alteration of macrophage phenotype or polarization is associated with distinct gene expression profiles (15).

Herein, we summarize the latest findings of new macrophage markers in different diseases. Firstly, the phenotypes of macrophages are briefly discussed. Secondly, some macrophage-associated molecules are selected to discuss their important roles in diseases, especially with regard to newly identified functions. Then, the potential application of some molecules as diagnostic markers or therapeutic targets for treatment is reviewed. Finally, the methods that are applied to deplete macrophages are discussed.

## MACROPHAGE PHENOTYPES

Macrophages are very plastic cells with different phenotypes and functions (Figure 1), which are impacted both by their origin and resident tissue microenvironment. Broadly, macrophages can be activated into two distinct subsets based on the M1/M2 paradigm, classically activated or M1 macrophages and alternatively activated or M2 macrophages (16). M1 macrophages are polarized *in vitro* by Th1 cytokines such as colony-stimulating factor (GM-CSF), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), and interferon- $\gamma$  (IFN- $\gamma$ ) alone or together with lipopolysaccharide (LPS) from bacteria. M1 macrophages express pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-12, IL-23, and TNF- $\alpha$  (17). In contrast, M2 macrophages are polarized by Th2 cytokines such as IL-4 and IL-13 and produce anti-inflammatory cytokines such as IL-10 and transforming growth factor beta (TGF- $\beta$ ) (18).

Recent studies have revealed that the M1/M2 paradigm is not sufficient to encompass all states of macrophage activation. Macrophages have the ability to change their polarization in response to different stimuli. For example, macrophage phenotype changes during tissue repair, switching from pro-inflammatory phenotype (M1-like) to an anti-inflammatory phenotype (M2-like) (19). In addition, different polarization and activation markers of M1- and M2-like macrophages can coexist in tissues (20). For instance, a high percentage of circulating macrophages expressing both M1 (CD80, CD86, and TLR4) and M2 surface markers (CD204, CD163, and CD206) was shown in human patients with interstitial lung disease (21). With the analysis of the transcriptomic profiles of macrophages, Liu et al. (22) reported that polarized M1- or M2-like macrophages

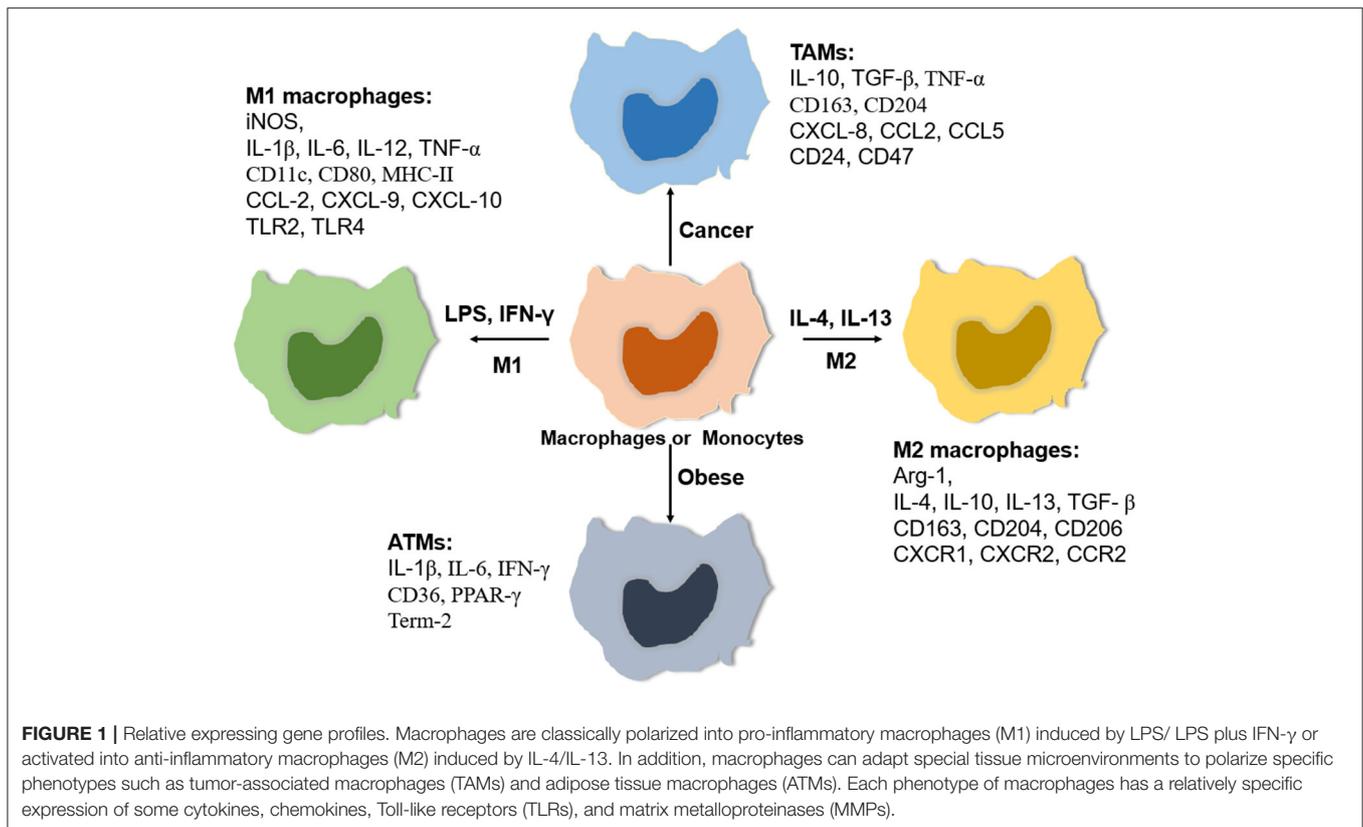
driven by cytokines can be subsequently repolarized to another phenotype with little or no memory of polarization history. Therefore, the *in vivo* phenotype and function of macrophages remains to be defined under specific tissue microenvironments.

Phenotypic change and functional polarization of macrophages are accompanied by a change in cellular metabolism, as M1-like macrophages primarily rely on glycolysis, whereas M2-like macrophages rely on oxidative phosphorylation (23). Parallel analysis of macrophage metabolic and transcriptional profiles also indicates that metabolic reprogramming impacts macrophage polarization or activation (24). For example, inhibition of aspartate-aminotransferase and N-glycosylation interfere with M1 and M2 macrophage polarization, respectively. Factors that affect macrophage metabolism may disrupt M1/M2 homeostasis. Hill et al. (25) reported that multiple distinct populations of adipose tissue-associated macrophages (ATMs) are present in adipose tissues in mice and humans, existing with unique transcriptomes, chromatin landscapes, and functions. Similarly, pro-inflammatory ATMs in obese mice or humans can express additional markers of metabolic activation distinct from the classical markers of activation (26).

In addition to the abilities of engulfing and digesting foreign pathogens and cellular debris, macrophages can clear away tumor cells. Tumor-associated macrophages (TAMs) are particularly abundant immune cells in cancer and exert strong influences on tumor initiation, progression, and metastasis (27, 28). Besides, TAMs can secrete different cytokines such as IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ) to suppress T cell-dependent antitumor function (29). TAMs can be polarized into pro-inflammatory (M1-like) phenotype and anti-inflammatory (M2-like) phenotype under the stimuli of different tumor microenvironments, with the majority of TAMs functioning as M2-like macrophages (30). Notably, differentially polarized TAMs may exert opposite effects on tumor development. For example, high numbers of infiltrating M2-like macrophages in patients with gastric cancer (GC) were associated with a low rate of overall survival (OS), while an elevated number of M1-like macrophages was associated with better OS (31). Using scRNA-seq data and cell trajectory analysis, Landry et al. (32) reported that core TAMs evolve toward a pro-inflammatory state in human glioblastoma, while peripheral TAMs develop an anti-inflammatory phenotype. Therefore, an accurate understanding of the function of TAMs is likely to advance cancer immunotherapy.

## IMPORTANT MOLECULES MEDIATING MACROPHAGE FUNCTION

Currently, scRNA-seq is a critical tool to investigate macrophage heterogeneity and function. For example, transcriptional profiling analysis showed that there are five clusters of alveolar macrophages (AMs) across homeostasis, acute inflammation, and resolving inflammation, all expressing macrophage-specific markers, such as CD68 and Lgals3 (Galectin-3). Among them, two clusters express tissue-resident airspace macrophage marker



genes, such as *Mrc1* (CD206) and *Itgax* (CD11c), and another three clusters with significant upregulation of recruited AM marker genes, such as *CD14*, *Ly6c1* (*Ly6c*), and *Sell* (*L-selectin*) (33). In addition, resident AMs show higher expression of M2-like macrophage genes and two clusters of recruited AMs have higher expression of M1-like macrophage genes, while the last cluster of recruited AMs exhibit relatively low expression of both M1-like and M2-like gene expression profiles. Overall, these data further indicate that the M1/M2 paradigm is not sufficient for classifying macrophage polarization. In this section, we highlight some important genes found in macrophage function and transcriptional profiling studies.

## CD163

The hemoglobin scavenger receptor (CD163) is a macrophage-specific protein (34), highly expressed on TRMs but modestly expressed on monocyte-derived macrophages. The expression of CD163 was up-regulated on human blood monocytes following stimulation with macrophage colony-stimulating factor (M-CSF), and down-regulated following stimulation with GM-CSF and IL-4 (35). In addition, CD163 expression was also suppressed by pro-inflammatory molecules and cytokines such as LPS, IFN- $\gamma$ , and TNF- $\alpha$ , and upregulated by anti-inflammatory cytokine IL-10, indicating CD163 is expressed on macrophages with the anti-inflammatory phenotype (35). Recent findings show that CD163<sup>+</sup> macrophages promote

tumor progression. Shiraishi et al. (36) reported that CD163<sup>+</sup> macrophages were associated with decreased overall survival of patients with pleomorphic sarcoma. Silencing CD163 abrogated macrophage-induced tumor cell proliferation in co-cultured cells of human monocyte-derived macrophages and leiomyosarcoma and myxofibrosarcoma cell lines. *In vivo* assays demonstrated the growth of sarcoma was significantly inhibited in CD163-deficient mice compared to wild-type mice, which was associated with the production of IL-6. In breast cancer, CD163-expressing TAMs resembling an M2-like phenotype accumulated in the tumor microenvironment and were associated with poor clinical outcomes (37). A similar difference is also found in colorectal tumors (CRC). The anti-inflammatory CD163<sup>+</sup> macrophages (M2-like) were more prevalent in advanced tumor stage and exclusively located in invasive tumor front, whereas the CD80<sup>+</sup> macrophages (M1-like) were predominant in less invasive tumors and predominantly distributed in tumor-adjacent normal mucosa (38). Moreover, a low CD80/CD163 ratio was associated with decreased overall survival in patients with CRC (39).

## LYVE-1

Lymphatic vessel hyaluronan receptor-1 (LYVE-1), the major receptor of hyaluronan (HA) in lymphatic vessel endothelial cells, is closely related to the leukocyte HA receptor CD44 (40), mediating the trafficking of leukocytes, including macrophages. Lim et al. (41) reported that LYVE-1<sup>+</sup> macrophages line

murine and human blood vessels, modulated collagen production in smooth muscle cells to maintain arterial wall homeostasis. Another study also showed LYVE-1<sup>+</sup> cells expressing macrophage markers CD68 or CD169 that co-localized with collagen fibers in rat meninges, and some LYVE-1<sup>+</sup> cells had intracellular collagen (42). Dollt et al. (43) also reported that extracellular domains of LYVE protein from M2-like macrophages significantly inhibited human and murine melanoma cell proliferation by acting as a receptor for low-molecular weight HA. In addition, the LYVE-1<sup>+</sup> macrophages have been shown to play critical roles in tissue remodeling (44) and murine eyes (45).

## MerTK

Synovial tissue macrophages (STMs) play critical roles in autoimmune diseases including rheumatoid arthritis (RA) (46). With the analysis of integrated scRNA-seq, deep-phenotypic, spatial and functional data, Alivernini et al. (47) found that synovial tissue macrophages (STMs) can be broadly classified into two populations, MerTK<sup>-</sup>CD206<sup>-</sup> STMs and MerTK<sup>+</sup>CD206<sup>+</sup> STMs. MerTK<sup>-</sup>CD206<sup>-</sup> STMs produce pro-inflammatory cytokines and alarmins and induce inflammatory responses in synovial fibroblasts, while MerTK<sup>+</sup>CD206<sup>+</sup> STMs from patients with RA in sustained disease remission produce lipid mediators that resolve inflammation and induce a repair phenotype of fibroblast-like synoviocytes. Also, they found that two STM subpopulations (MerTK<sup>+</sup>TREM2<sup>high</sup> and MerTK<sup>+</sup>LYVE1<sup>+</sup>) with unique remission transcriptomic signatures, enriched in negative regulators of inflammation (47). Kuo et al. (48) also identified a subset of inflammatory macrophages expressing heparin-binding EGF-like growth factor in RA joints, which altered synovial fibroblast gene expression profile (e.g., IL-33) via up-regulation of epidermal growth factor receptor (EGFR) response and increased their invasiveness (47, 48). MerTK as a key efferocytosis receptor, is primarily expressed by CD11b<sup>+</sup> F4/80<sup>+</sup> large peritoneal macrophages (LPMs). At steady-state, MerTK-deficient LPMs exhibit significantly increased pro-inflammatory cytokine expression, when under stimulation of apoptotic cells, MerTK<sup>-/-</sup> LPMs increased gene expression of cell death and apoptosis (49).

## SIGLECS

Siglecs (sialic acid-binding immunoglobulin-type lectins) are transmembrane surface proteins found primarily on hematopoietic cells, consisting of intracellular tyrosine motifs involved in cell signaling (50). Siglecs predominantly recognize sialic acid residues of glycoproteins on the cell membrane. Fourteen of 15 known Siglecs are present in humans, namely Siglec-1 to 15 except Siglec-13 which is present in nonhuman primates. Siglecs on macrophages or monocytes play critical roles in many different diseases, as described in **Table 1**. Siglecs appear to have predominantly pro- or anti-inflammatory functions in macrophages. For example, Siglec-1, a receptor on monocytes/macrophages, plays a central role in the pathogenesis

of congenital heart block and contributes to IFN stimulation (51). Plasma concentrations of Siglec-1 are also strongly correlated with type I interferon-regulated gene expression in systemic lupus erythematosus (SLE) patients (75), suggesting a pro-inflammatory influence.

In the context of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), liver Kupffer cells and recruited monocyte-derived macrophages play pivotal roles in the pathophysiology of NAFLD and NASH (76). In NAFLD patients, Siglec-7 was mostly expressed in hepatic CCR2<sup>+</sup> macrophages, in contrast, its expression was much weaker in resident macrophages (58). In addition, soluble Siglec-7 was shown highly produced in monocyte-derived macrophages and serum sSiglec-7 can be used as an independent marker for NAFLD in patients with advanced liver fibrosis (58).

Siglec-9, as a mediator of inflammation, is a potential target for the treatment of sepsis. Pretreatment with a human anti-Siglec-9 Fab fragment attenuated LPS-induced pro-inflammatory cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$  production in human peripheral blood mononuclear cell (PBMC)-derived macrophages and human THP-1-differentiated macrophages (60). Following exposure to the sialic acid-expressing human bacterial pathogen group B *Streptococcus* (GBS), Siglec-14, as a positive regulator of NLRP3 inflammasome activation on macrophages, resulted in the release of the pro-inflammatory cytokine IL-1 $\beta$ . Mononuclear phagocytes are also attractive drug delivery vehicles for novel cancer treatment owing to their cancerous tissue-accumulating nature. Given Siglecs are highly expressed in TAMs and peripheral blood monocytes, sialic acid-conjugated liposomes have been applied to deliver tumor-targeting drugs (77). Moreover, other Siglecs including Siglec-2 (52), Siglec-3 (53), Siglec-4 (54), Siglec-5 (55, 56), Siglec-6 (57), Siglec-8 (59), Siglec-10 (61), Siglec-11 (62), Siglec-12 (63), Siglec-15 (64) also play important roles in diseases as listed in **Table 1**.

## SIRP $\alpha$

CD47, known as the “don’t-eat-me” signal, is commonly overexpressed on the surface of tumor cells (78, 79). Signal regulatory protein  $\alpha$  (SIRP $\alpha$ ) on macrophages serves as a receptor of CD47 to transduce CD47/SIRP $\alpha$  axis mediated inhibitory function of macrophage phagocytosis of tumor cells (80). Blockade of CD47-SIRP $\alpha$  signaling is a strategy to promote macrophage phagocytosis of many cancer cells, which is discussed in the therapeutic section. A number of preclinical and clinical investigations are underway to target the CD47/SIRP $\alpha$  axis for cancer therapy, including examination of the synergistic effect with other anti-tumor agents such as rituximab (anti-CD20 antibody), cetuximab (an inhibitor of EGFR), and trastuzumab (an inhibitor of human epidermal growth factor receptor 2, HER2) (81).

## TREM2

The prevalence of obesity has reached epidemic levels, and over 44% of adults are overweight worldwide. Obesity substantially

**TABLE 1** | The role of Siglecs in macrophage function.

Siglecs	Diseases	Function	References
Siglec-1 (CD169)	Congenital heart block (CHB)	The expression of IFN and type I IFN-stimulated genes, including Siglec-1, a receptor on monocytes/macrophages, play an important role in the pathogenesis of congenital heart block.	(51)
Siglec-2 (CD22)	Aging brain	CD22 (siglec-2) mediated the anti-phagocytic effect and inhibition of CD22 promoted the clearance of myelin debris, amyloid- $\beta$ oligomers, and $\alpha$ -synuclein fibrils <i>in vivo</i> . A long-term CNS-delivery of a CD22 blocking antibody activated microglia, resulting in the improvement of cognitive function in aged mice.	(52)
Siglec-3 (CD33)	Alzheimer's disease	Deletion of hCD33 in macrophage cell lines U937 and THP-1 increased cargo uptake <i>in vitro</i> . In addition, transgenic mice expressing hCD33 in the microglial cell lineage showed inhibited cargo uptake in primary microglia.	(53)
Siglec-4 (Myelin-associated Glycoprotein, or MAG)	CNS pathology	Myelin-associated glycoprotein (MAG), a minor constituent of central and peripheral nervous system myelin, binds to gangliosides GD1a and GT1b, prominent molecules on the axon surface, mediating axon stability in the central nervous system and peripheral nervous system.	(54)
Siglec-5 (CD170)	Asthma	Siglec-5 expression was significantly increased in patients receiving inhaled corticosteroids, exerting a beneficial effect. Double staining of cells indicated that Siglec-5 was expressed in monocyte/macrophages and neutrophils, but not in lymphocytes. Exposure to the sialic acid-expressing human bacterial pathogen group B <i>Streptococcus</i> (GBS), Siglec-5 negatively regulates inflammation.	(55, 56)
Siglec-6 (CDw327)	Colorectal cancer (CRC)	The siglec-6 expression on mast cells is involved in their function in the tumor microenvironment of CRC, but no evidence shown in macrophages.	(57)
Siglec-7 (CDw328)	Non-alcoholic fatty liver disease (NAFLD)	Serum Siglec-7 could serve as an independent marker for advanced liver fibrosis in patients with NAFLD.	(58)
Siglec-8 (Siglec-F in mouse)	Lung disease	Therapeutic targeting of Siglec-8 has the potential to impact blood as well as lung eosinophils, which may be associated with an improved outcome in eosinophilic lung diseases.	(59)
Siglec-9 (CD329 and Siglec-E in mouse)	Sepsis	Blockade Siglec-9 induced inflammation by anti-Siglec-9 Fab fragment (hS9-Fab03) is a potential therapeutic agent for sepsis.	(60)
Siglec-10 (Siglec-G in mouse)	Ovarian and breast cancers	Blockade of CD24-Siglec-10 signaling is a potential therapeutic strategy for breast and ovarian cancer immunotherapy.	(61)
Siglec-11	Neural disease	The polysialic acid (polySia) with an average degree of polymerization 20 (avDP20) neutralized the LPS-triggered increase in macrophage phagocytosis, by interacting with SIGLEC-11.	(62)
Siglec-12 (Siglec-like molecule-1)	Prostate cancer	The stable expression of Siglec-12 enhanced prostate cancer cell growth in nude mice. Anti-Siglec-12 monoclonal antibodies were internalized by Siglec-12-expressing prostate carcinoma cells, providing a target.	(63)
Siglec-14	Bacteria	Siglec-14 enhances NLRP3 inflammasome activation in macrophage, in response to known inflammasome activators or the sialic acid-expressing human bacterial pathogen GBS.	(56)
Siglec-15 (Siglec-H in mouse)	Cancer	Siglec-15 on macrophages may contribute to tumor progression by targeting the sialyl-Tn (sTn) antigen, a tumor-associated glycan structure, which modulating TGF- $\beta$ secretion in tumor microenvironments.	(64)

increases the risk of many diseases, including type 2 diabetes mellitus, NAFLD, hypertension, myocardial infarction, stroke, osteoarthritis, and cancer (82). Recently, scRNA-seq analysis of the immune cells in murine lipid-associated macrophages (LAMs) (83), NASH-associated macrophages (84), and aortic macrophages (85) indicated that a subset of macrophages expressing triggering receptor expressed on myeloid cells 2 (TREM2), which drives a gene expression program involved in phagocytosis, lipid catabolism, and energy metabolism. Further study showed that TREM2<sup>+</sup> macrophages arose from circulating monocytes and positioned around enlarged

adipocytes and scRNA-seq data of human adipose tissue indicated that LAM cells, as well as the TREM2 pathway, were highly conserved (83). TREM2 deletion abrogated macrophage recruitment to enlarged adipocytes and caused massive adipocyte hypertrophy, systemic hypercholesterolemia, inflammation, and glucose intolerance, indicating the protective role of TREM2<sup>+</sup> macrophages (83). TREM2<sup>-/-</sup> mice fed a high-fat diet (HFD) exhibited a reduction of infiltrating F4/80<sup>+</sup>CD11c<sup>+</sup> macrophages in adipose tissue but displayed augmentation of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, and inducible nitric oxide synthase (iNOS), adipocyte hypertrophy,

hepatic steatosis, and insulin resistance compared with WT controls (86).

TREM2 expression promotes the transition of liver macrophages from the pro-inflammatory to the tissue repair phase and impacts endothelial cell differentiation during tissue recovery in mouse models of acetaminophen (acute) or CCL<sub>4</sub> (chronic)-induced hepatotoxic injury (87). In addition, TREM2 expression prevents lung macrophage apoptosis during acute parainfluenza virus infections. A more active soluble form of TREM2 (sTREM2) was found after clearance of infection, which is unexpectedly active in preventing macrophage apoptosis (88).

However, the function of TREM2 may change according to disease stage and location, with several studies suggesting CNS-specific functions of TREM2. Recent genetic studies show that TREM2 mutation is also associated with a higher risk of Alzheimer's disease (AD) and multiple neurodegenerative disorders (89). In mice, TREM2 deficiency enhanced macrophage activation near the lesion of traumatic brain injury (TBI) but significantly reduced macrophage activation distant from the lesion compared to control groups in the acute stage (90). In addition, TREM2 deficiency resulted in a protective function at later time points, as the TREM2<sup>-/-</sup> mice showed a reduction of hippocampal atrophy and rescue of TBI-induced behavioral changes compared to wild-type mice.

## OTHERS

In addition to the molecules as described above, there are several less well-studied molecules expression in macrophages, including osteopontin (encoded by gene *Spp1*) (12), CLEC2 (C-type lectin domain family 1 member B, encoded by gene *Clec1b*) (91), and CD48 (92). Overall, understanding the molecular mechanism of macrophages in tissue microenvironment is critical to design macrophage-mediated therapy in diseases.

## PROGNOSTIC MARKERS

Two of the best studies on macrophage-associated prognostic markers include soluble CD163 (sCD163)<sup>+</sup> and CD204<sup>+</sup> TAMs, with particular utility in the context of liver disease and various cancers, respectively. Soluble CD163 (sCD163) is upregulated during macrophage proliferation and activation and is a marker for diagnosis of the severity and progression of liver disease. For instance, Lidofsky et al. (65) reported that serum sCD163 from macrophages in patients infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) was positively associated with the severity of liver fibrosis from mild to moderate stage, with an Ishak fibrosis score up to 4, but not in established cirrhosis. These results suggest that sCD163 is a dynamic biomarker of hepatic fibrogenesis rather than cirrhosis in patients with viral infections (65). Another study found that sCD163 was associated with primary biliary cholangitis disease severity and long-term (a median of 8.6 years) prognosis, following a study in 201 patients (66). Besides, using sCD163 as a marker increased the prediction accuracy of poor outcomes of PBC. The sCD163 levels were also elevated in patients with early

allograft dysfunction after liver transplantation, as macrophages were activated in the implanted liver when exposed to ischemia and reperfusion injury (67).

Accumulating evidence reveals that CD204<sup>+</sup> TAMs promote cancer cell proliferation, invasion, and metastasis, resulting in a poor survival rate. Kawajiri and colleagues reported that a high number of CD204<sup>+</sup> TAMs was associated with poorer 3-year overall survival (OS) and cumulative incidences of relapse, and a poorer prognosis in allogeneic hematopoietic cell transplantation for malignant lymphomas, such as T-cell lymphoma and leukemia (68). In lung adenocarcinoma, the expression of CD204 in TAMs was associated with a low 5-year disease-free survival rate and the aggressiveness of lung adenocarcinoma (69). CD204<sup>+</sup> TAMs located in the tumor stroma area were also identified as useful prognostic markers in non-small-cell lung cancer (NSCLC) (70) and an increased number of CD204<sup>+</sup> TAMs was positively associated with worse clinical prognoses in breast cancer, including relapse-free survival, distant relapse-free survival and breast cancer-specific survival (71). CD204 is also a useful marker for TAMs contributing to the angiogenesis, progression and prognosis of esophageal squamous cell carcinomas (72).

Dual markers CD163 and CD204 can also be used in diagnosis (Table 2). For instance, the numbers and percentages of M2-polarized alveolar macrophages expressing markers CD163<sup>+</sup>, CD204<sup>+</sup>, and CD206<sup>+</sup> increases with the severity of chronic obstructive pulmonary disease, with higher numbers in smoker patients than non-smokers (73). In clinical findings, the number of CD163<sup>+</sup>CD204<sup>+</sup> TAMs is negatively correlated with that of CD25<sup>+</sup> cells (presumably activated lymphocytes) and 5-year progression-free survival. Dual CD163<sup>+</sup>CD204<sup>+</sup> TAMs possibly play a vital role in the invasion and metastasis of oral squamous cell carcinoma by T-cell regulation via IL-10 and PD-L1 production, relative to CD163<sup>+</sup>CD204<sup>-</sup> TAMs or CD163<sup>-</sup>CD204<sup>+</sup> TAMs (74).

## THERAPEUTIC TARGETS FOR DISEASES

While M2-like pro-tumoral phenotype is dominant in tumor microenvironment, targeting TAMs or conversion of TAMs to an M1-like anti-tumoral phenotype is an emerging strategy for targeting TAMs-mediated cancer therapy (93). An *in vivo* assay showed that a hybrid peptide composed of melittin and the pro-apoptotic peptide, selectively targeted TAMs without impacting affecting other leukocytes, such as T cells and dendritic cells. Treatment of the hybrid peptide resulted in apoptosis of CD206<sup>+</sup> M2-like TAMs and reduction of tumor growth and angiogenesis, showing as a promising cancer therapeutic agent (94).

Targeting "Don't eat me" signaling pathway such as blocking the CD47-SIRP $\alpha$  interaction can improve macrophage phagocytosis of tumor cells. Monotherapy of TTI-621, a fusion protein consisting of CD47 binding domain of SIRP $\alpha$ , is well-tolerated and shows rapidly active responses in adult patients with relapsed/refractory percutaneously accessible solid tumors (95). Another study showed that anti-human SIRP $\alpha$

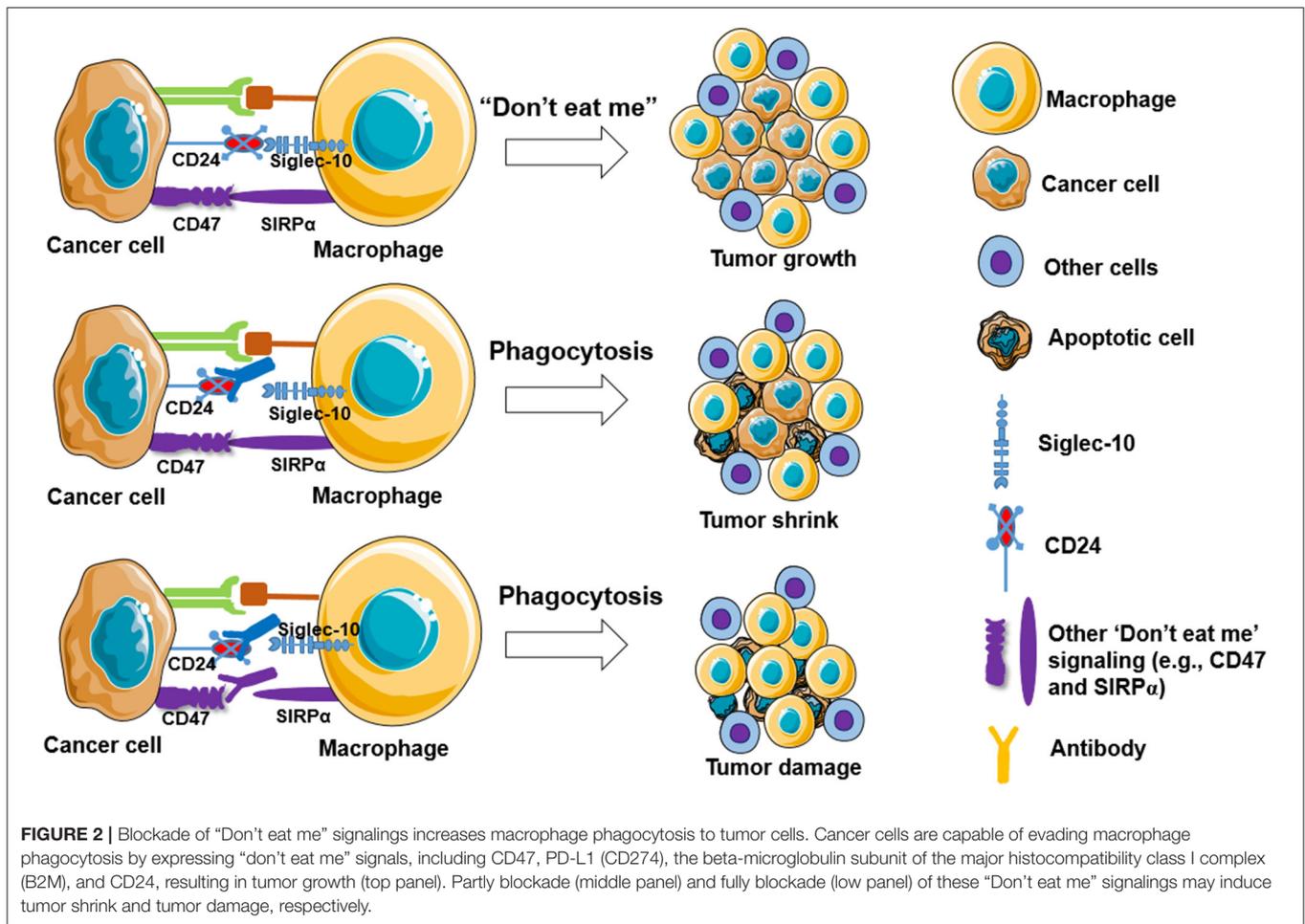
**TABLE 2** | Macrophage expressing markers CD163, CD204, or both in disease diagnosis.

Markers	Diseases	Function	References
CD163	Liver fibrosis	Under the coinfection with human immunodeficiency virus (HIV) or hepatitis C virus (HCV), serum (s)CD163 levels accompanied periportal CD163+ macrophage enrichment was associated with mild to moderate fibrosis, but not cirrhosis.	(65)
	Primary biliary cholangitis (PBC)	sCD163 represents a non-invasive measure of that provides useful long-term prognostic for PBC.	(66)
	Early allograft dysfunction (EAD)	The sCD163 levels in patients were also elevated in patients with early allograft dysfunction (EAD) after liver transplantation, as macrophages were activated in the implanted liver when exposed to ischemia and reperfusion injury.	(67)
CD204	Malignant lymphomas, such as T-cell lymphoma and leukemia	A high number of CD204+ TAMs was associated with 3-year poorer overall survival (OS) and cumulative incidences of relapse, and the poorer prognosis in allogeneic hematopoietic cell transplantation for malignant lymphomas.	(68)
	Lung adenocarcinoma	The expression of CD204 in TAMs was associated with a low 5-year disease-free survival (DFS) rate and the aggressiveness of lung adenocarcinoma. In lung adenocarcinoma, CD204+ TAMs located in the tumor stroma area were the preferable marker for prognostic prediction in non-small-cell lung cancer (NSCLC).	(69, 70)
	Breast cancer (BC)	An increased number of CD204+ TAMs was positively associated with worse clinical prognoses in breast cancer, including relapse-free survival, distant relapse-free survival and breast cancer-specific survival.	(71)
	Esophageal squamous cell carcinomas (ESCCs)	CD204 is a useful marker for TAMs contributing to the angiogenesis, progression and prognosis of esophageal squamous cell carcinomas (ESCCs).	(72)
CD163 and CD204	Chronic obstructive pulmonary disease (COPD)	The numbers and percentages of M2 markers CD163+CD204+ alveolar macrophages were positively associated with the severity of chronic obstructive pulmonary disease (COPD), with higher numbers in smoker patients than non-smokers.	(73)
	Oral squamous cell carcinoma (OSCC)	Dual CD163+CD204+ TAMs possibly play a key role in the invasion and metastasis of oral squamous cell carcinoma (OSCC) by T-cell regulation via IL-10 and PD-L1 production, more valuable than CD163+CD204- TAMs or CD163-CD204+ TAMs.	(74)

monoclonal antibody (Ab) KWAR23 treatment alone does not increase human macrophage phagocytosis of CD20-expressing lymphoma cells, but significantly augments phagocytosis of lymphoma cells when combined with treatment of anti-CD20 antibodies (e.g., rituximab) *in vitro*, and this effect was also shown in a lymphoma xenograft mouse model expressing human SIRP $\alpha$  (96). Murata et al. (97) recently showed that anti-human SIRP $\alpha$  Ab significantly enhances macrophage phagocytosis of lymphoma cancer cells in immunodeficient mice harboring a transgene of human SIRP $\alpha$ , which also shows a synergistic effect of rituximab on inhibition of tumor growth. In addition, anti-mouse SIRP $\alpha$  (P84 or MY-1) Ab treatment markedly inhibits the growth of tumors formed by SIRP $\alpha$ -expressing renal cell carcinoma and melanoma in mice. Moreover, the anti-SIRP $\alpha$  Ab treatment shows a synergistic effect in combination with rituximab and anti-programmed cell death 1 (PD-1) on tumor formation in mice (98). Other strategies have been applied to improve macrophage-mediated anti-tumor responses and reduce the toxicity to normal cells, such as using

exosomes harboring SIRP $\alpha$  to block the interaction between CD47 and SIRP $\alpha$  (99) and augmenting the contact interface of CD47-SIRP $\alpha$  via engineering N-terminal peptide extension (80). In addition, new signaling pathways are also reported to function similarly. For example, Barkal et al. (61) reported that CD24 signaling through macrophage Siglec-10 is a potential therapeutic target for cancer immunotherapy (**Figure 2**), as blockade of CD24-Siglec-10 signaling can promote macrophage phagocytosis of triple-negative breast cancer and ovarian cancer cells (61).

Cai et al. (100) reported that MerKT in liver macrophage contributes to liver fibrosis since MerKT-deficient mice slowed the development of liver fibrosis compared to wild-type mice when fed a NASH-promoting diet. Molecular study showed an increase of MerKT expression on macrophages induced TGF- $\beta$ 1 expression and secretion via ERK1/2 signaling, which promoted hepatic stellate cell activation to produce collagen production. The authors also found that blockade of the interaction of MerKT with its ligand Gas6 using RU-30, an inhibitor of receptors



(Tyro-3, Axl, and Mertk), significantly inhibited the production of collagen.

## STRATEGIES TO STUDY THE FUNCTION OF MACROPHAGES

To study the precise functions of macrophages under different physiological and pathological conditions, several strategies have been used to deplete macrophages in various mouse models, including genetic ablation (101), nanotechnology-based depletion by clodronate-encapsulated liposomes (102), and antibody-mediated depletion (103). These strategies have produced encouraging results in experimental murine models. For example, in transgenic mice based on CD11b promoter-mediated expression of the human diphtheria toxin (DT) receptor, transient depletion of macrophages occurs *in vivo* when DT is injected. Using this model, the researchers identified that functionally distinct subpopulations of macrophages exist in liver injury and recovery, inducing liver fibrosis by promoting extracellular matrix deposition during liver injury or promoting the resolution of liver fibrosis during recovery (104). These

methods are however not perfectly specific for macrophages and induce only temporary ablation.

Clodronate-encapsulated liposomes have been broadly applied to deplete macrophages in different tissues (bone marrow, spleen, liver, lungs, brain, gut, peritoneal cavity, lymph nodes/vessels) and blood. A protocol used to deplete macrophages in mice was described before (102). For instance, a single i.p. injection of clodronate liposome during chronic cholangiopathy resulted in significant inhibition of ECM deposition in mice (105). In a murine model of Azoxymethane (AOM)/Dextran Sodium Sulfate (DSS)-induced colorectal cancer, mice received multiple treatments with clodronate to deplete macrophages resulted in a significant decrease of tumor number by ~35%, specifically for large ( $\geq 1$  mm) tumors. This effect was accompanied with a decrease in gene expression of pan macrophage marker F4/80 as well as expression of markers associated with M2 macrophages (IL-13, IL-10, TGF- $\beta$ , and CCL17) (106). In addition, macrophage depletion was also associated with a significantly increased relative abundance of the *Firmicutes* phylum in stool, demonstrating that macrophages are important mediators of tumor growth and directly or indirectly capable of influencing the gut microbiota during colorectal cancer development (106). TAMs increased as urethane-induced

lung tumor grew in mice, which exhibited a mixed M1/M2-like macrophage phenotype. Liposomal clodronate treatment (one time of intrathecal administration plus five times of i.v. administration) significantly decreased the alveolar macrophage population (> 50%) and resulted in a 50% reduction in tumor burden compared to vehicle liposome-treated mice (107). A subsequent study demonstrated that inhibition of recruited macrophages by blockade of CCL2/CCR2 signaling did not attenuate lung cancer progression, as CCR2-deficient mice showed similar tumor growth rate compared to wild-type mice.

However, depletion of the entire macrophages in a specific tissue or whole body may result in unwanted side effects. For instance, depletion of peritoneal macrophages with clodronate injection induced a dramatic decrease in neutrophil recruitment in experimental peritonitis (108). Another study showed transient depletion of macrophages may exacerbate liver injury in NASH patients since monocytes-derived KCs show more pro-inflammatory activity and are less efficient in hepatic triglyceride storage (91). As we discussed above molecular markers, some subpopulations of macrophages also benefit the resolution of diseases, such as LYVE-1+ macrophages. Thus, deletion of specific macrophage subpopulation or specific gene in macrophages under investigation may represent a more reasonable approach.

## SUMMARY

Macrophages are a key population of innate immunity, with powerful influences on homeostasis, tissue repair, obesity, and cancer. Macrophages consist of two populations,

tissue-resident macrophages with a prenatal origin and postnatal monocyte-derived macrophages. Independent of their origin, macrophages are very plastic cells, being able to change their phenotype according to local environmental stimuli. They are usually polarized into M1-like or M2-like phenotype; however, recent studies suggest that the phenotype of macrophages cannot be simply divided into M1/M2 dichotomy, and additional classifications, such as TAMs and ATMs, are applied to define the tissue-specific macrophages. In this review, promising candidate macrophage markers are highlighted due to their potential application in diagnosis and treatment against diseases.

Several methods have been applied to target TAMs or ATMs to prevent tumor growth or inflammation, such as macrophage depletion, blockade of anti-phagocytic signaling (e.g., Siglec-10 or SIRP $\alpha$ ). Therefore, understanding the molecular mechanisms of macrophage function in disease provides potent diagnostic markers and/or therapeutic strategies.

## AUTHOR CONTRIBUTIONS

CZ and MY conceived and wrote the manuscript. AE provided guidance for part of manuscript, critically reviewed and significantly edited the manuscript. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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