SERAFINO ZAPPACOSTA AND THE CEPPELLINI SCHOOL: A PIONEER MODEL FOR NURTURING EDUCATION IN IMMUNOLOGY

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SERAFINO ZAPPACOSTA AND THE CEPPELLINI SCHOOL: A PIONEER MODEL FOR NURTURING EDUCATION IN IMMUNOLOGY

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This Research Topic is dedicated to the memory of Ennio Carbone (1961-2020).

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EDITORIAL
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Editorial: Serafino Zappacosta and the Ceppellini School: A Pioneer Model for Nurturing Education in Immunology

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Editorial on the Research Topic

Serafino Zappacosta and the Ceppellini School: A Pioneer Model for Nurturing Education in Immunology

This *Frontiers in Immunology Research Topic* is a collection of articles on the activities and the scientific interests of the founders, faculty, and students of the "Scuola Superiore di Immunologia Ruggero Ceppellini" (Ruggero Ceppellini Advanced School of Immunology), an International School of Immunology founded almost 30 years ago following a pioneer idea by Serafino Zappacosta. The school has more recently become known as the EFIS-EJI Ruggero Ceppellini Advanced School of Immunology founded by Serafino Zappacosta. The re-naming of the school followed the sudden death of Zappacosta in 2006 (1). Furthermore, in 2011 the European Federation of Immunological Societies (EFIS) declared the Ceppellini School one of its regularly sponsored activities. Since then, the European Journal of Immunology (EJI, i.e., the EFIS official journal) has regularly reported on the Ceppellini School's international courses in its "News & EFIS" section [for recent examples see (2–4)].

The opening article of this *Research Topic* is a contribution by Antonio Di Giacomo (Colli Monaldi Hospital, Naples, Italy) who, in 1991, joined Zappacosta (at the time a full professor of immunology at the Federico II University, Naples) in the foundation of the Ceppellini School in Naples, Italy (Di Giacomo). Co-founders were Melchiorre Brai (University of Palermo, Italy), Giovanni B. Ferrara (Federico II University, Naples), Ciro Manzo (Istituto Pascale, Naples), and Alfred Nisonoff (Brandeis University, in Waltham, Massachusetts, USA). The title of Di Giacomo's article, "The Ruggero Ceppellini Advanced School of Immunology and the Neapolitan Scientific Renaissance," clearly indicates the strong roots of the Ceppellini School in the city of Naples. Di Giacomo illustrated the pioneer vision of the founders and their strong commitment to the generous educational project of the Ceppellini School, which was summarized in the Latin motto suggested by Zappacosta "*non multa sed multum*" (not many but much, i.e., quality not quantity) (Di Giacomo).

The second article is a tribute to Zappacosta by a group of previous students and collaborators who worked with him in Naples, all of them now well-established immunologists in Italy, Europe, and the USA (Carbone et al.). They reported on the research performed by Zappacosta and his team over more than 30 years on the role of MHC in innate and adaptive immunity, showing how their findings contributed to, and often anticipated, key issues of current literature. Silvia Fontana, one of the authors of this perspective, became the President of the Ceppellini School after Zappacosta's death, and her strong commitment and passionate work have been essential for the continuation of the School's educational project. The first author is Ennio Carbone, co-editor of this Research

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Topic, who sadly died all of a sudden in March 2020 just after he became President of the Ceppellini School. We co-edited this *Research Topic*, but, unfortunately, he could not co-author this editorial. This *Research Topic* is dedicated to his memory.

Zappacosta and colleagues entitled their Advanced Immunology School to Ruggero Ceppellini, an outstanding Italian scientist who gave seminal contributions to the genetics of HLA. Here, Walter Bodmer (University of Oxford, UK) drew a picture of Ruggero Ceppellini and reported about some of his achievements and fruitful insights that inspired his contemporary colleagues and those who followed his path in later years (Bodmer). The inaugural course of the Ceppellini School was on bone marrow transplantation (BMT) in 1992. It was directed by Elizabeth Simpson, at the time working at the Division of Transplantation Biology, MRC Clinical Research Centre, Harrow, Middlesex, UK. In their article for this *Research Topic*, Elizabeth Simpson and Francesco Dazzi (King's College, London, UK) placed the achievements of about six decades of research and clinical experience on BMT in the context of today challenges and discussed how the contributions to the 1992 Ceppellini School course created a remarkable marker point about mid-way between the first BMT in 1957 and current times (Simpson and Dazzi).

In 2006, Stefan Kaufmann, who was Director of the Max-Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany, became Scientific Director of the Ceppellini School. Kaufmann organized and directed several Ceppellini School courses, mostly focused on immune response and vaccination against tuberculosis and other threatening infectious diseases, such as malaria and AIDS (5). Here, Kaufmann gave a historical overview of the most remarkable milestones in immunology, focused on the Nobel laureates' achievements (Kaufmann). This personal and passionate perspective concisely summarized an overwhelming body of work. We also published a commentary by Heniz Kohler (University of Kentucky, Lexington, KY, USA) and colleagues, who integrated Kaufmann's review by emphasizing some additional aspects, for example, the theorical contribution of the idiotypic network theory by Jerne, the thoughtful work on positive and negative selection (of both T and B cells), and the current successes of therapeutic antibodies (Kohler et al.).

Rino Rappuoli, a distinguished vaccinologist who has been part of the faculty of many Ceppellini School courses over the years (5), is co-author, alongside Emanuele Andreano, Ugo D'Oro, and Oretta Finco, of a mini-review discussing the most promising approaches to vaccinology, going from the genome-based "reverse vaccinology" at the end of last century to the "reverse vaccinology 2.0" in 2016 and beyond (Andreano et al.). Siamon Gordon (University of Oxford, UK), Stefan Kaufmann, and Fernando Martinez-Estrada (University of Surrey, UK) were the scientific directors of a memorable Ceppellini School course on tissue phagocytes and function held in 2016 at the Stazione Zoologica "Anton Dohrn," a research center in Naples where the Russian scientist Elie Metchnikoff (1845–1916), who first described phagocytosis, worked for a short while (2). The contribution by Siamon Gordon and Annette Plüddemann (University of Oxford, UK) to this *Research Topic* is an inspiring discussion on macrophages diversity and function that highlights key open questions on macrophage heterogeneity and provides insights on its underlying pattern (Gordon and Plüddemann).

The last two articles focus on the fruitful sharing of knowledge between young attendees and senior faculty members of some exemplary Ceppellini School courses (4, 6). One article is by Francesco Colucci (University of Cambridge, UK), a Ceppellini School faculty member who was scientific co-director of the 2014 course on the maternal immune system in pregnancy (Colucci). The other article is by three of the participants to the 2018 Ceppellini School course on T-cell memory, i.e., Silvia Piconese (Sapienza University, Rome, Italy), Silvia Campello (University of Rome Tor Vergata, Rome, Italy), and Ambra Natalini (Sapienza University, and Institute of Molecular Biology and Pathology, CNR, Rome, Italy) (Piconese et al.). Both articles give a flavor of the exceptional learning experiences of participants to the Ceppellini School activities.

In 1991, the foundation of the Ceppellini School was a real breakthrough. After almost 30 years, the Ceppellini School continues to be an attractive pole for hundreds of young and enthusiastic participants from Europe, North and South America, the Middle East, Africa, and India. This *Research Topic* aims to offer some historical background and insightful perspectives on the Ceppellini School. Born from a Zappacosta's utopian idea, the school remains dedicated to strongly engaging new generation of young minds.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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The Ruggero Ceppellini Advanced School of Immunology and the Neapolitan Scientific Renaissance

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In this article the author, cofounder with Serafino Zappacosta and few other knowledgeable scientists of the Ruggero Ceppellini Advanced School of Immunology in 1991, discusses the significance of this initiative not only for the spreading of immunological culture among scientists—including those from disadvantaged Countries—but also for the resurgence of the city of Naples as a cultural pole of attraction for brilliant minds, as it was in its past history. This is a tribute to Serafino Zappacosta's foresightedness and generosity.

Keywords: school, Serafino Zappacosta, immunology, renaissance, Naples (Italy)

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Great intellectual achievements are the excellent fruits of rich and stimulating environments, fertile soils that prepare and nurture the mind. Roses do not bloom in a desert.

In this respect, culture constitutes the background for the development of new ideas and discoveries, that in every field of human knowledge represent the tools of advancement and innovation.

Biological sciences, intended as the study of the significance of the processes of life and not only their mechanical aspects, share with the human sciences the vast realm of the thinking mind, since when man experienced what Teilhard de Chardin defined as "the first moment" of self-awareness. Questions started to appear and science was born.

These and other related considerations was I debating in my thoughts when I met professor Serafino Zappacosta in a gray afternoon of November 1988 in the venues of a course on "Immunity in human pathology" that he used to give every 5 years at the Medical Faculty of the University of Naples "Federico II" where he was tenure professor of Immunology.

Indeed I had known him and his fame from before, as a medical student of that Faculty, always attracted by his charismatic personality and by the matter of his teaching, Immunology, at that time still a fast growing science, but I never had the opportunity of joining his group, nor did I try, the development of my career bringing me elsewhere. On that occasion, however, he showed interest in my curriculum and my recent experience abroad in experimental Immunology, and invited me to give seminars in his Institute about experimental cancer immunology, the subject of my studies. A collaboration started at that point, as did a long friendship.

In those years professor Zappacosta was maturing the intention to create a School of Immunology of international relevance in Naples, that would attract renowned scholars of that matter as teachers and an international audience of students coming from all over the world, in particular from developing Countries, in order to spread and promote immunological knowledge among scientists. He proposed me to join this project, along with a restricted group of scientists such as:

Melchiorre Brai, professor of Immunology at the University of Palermo; Giovan Battista Ferrara, professor of Human Genetics at the University of Naples "Federico II"; Albert Nisonoff, professor

of Biology at Brandeiss University, Mass. USA; and Dr. Ciro Manzo, head of the Immunology Department of the Istituto Pascale in Naples. I enthusiastically accepted, of course, honored and flattered by his invitation.

The School was founded in June 1991 with the name "Ruggero Ceppellini Advanced School of Immunology," dedicated to the memory of Ruggero Ceppellini (1917-88), the great Italian immunogeneticist, as a non-profit scientific association whose aim was to foster, encourage and propagate all aspects of knowledge relating to immunology and associated disciplines (genetics, microbiology, oncology) in the scientific community in Italy and in other Countries, through the promotion of scientific research, continuing education and in-service training.

The School's structure consisted in a Council of Directors and a Scientific Advisory Board, composed by a group of scientists each prominent in different areas of Immunology, that proposed and in turn took charge of the courses each year. Technically, the School teaching programmes were conceived and realized according to a "three level" scheme.

Level I courses, the typical refreshing courses, dealing with the so-called continuing education of medical graduates and designed to update the local practitioners on the recent advances having a bearing on their medical thinking and daily operation.

Level II courses, dedicated to young researchers working in Immunology or related fields, wishing to acquire knowledge of a specific topic within the vast area of Immunology. These are 1-week full-immersion activities, often integrated by workshops and small group discussions. Typical audience of these courses has been represented by Ph.D. students coming from all over the world.

Level III courses, short practical laboratory courses, dealing with recent techniques to be applied in research or even in the clinical laboratory, for small groups of graduates.

The School's inaugural ceremonies were held on 11 October, 1992, at Palazzo Serra di Cassano in Naples, the seat of the Istituto Italiano per gli Studi Filosofici, in the occasion of the School's first course, on the immunology of bone marrow transplantation. Many more courses followed, all successful and attended by students from all over the world, but more remarkable was the returning of Naples as the pole of attraction for scientific knowledge and culture after a long period of oblivion.

And this was the focal point of the all thing, the adventure clear to my mind since the beginning, the basic ideal drive that led me to join professor Zappacosta's dream to bring back to Naples the attention of the world's scientific community. Love for science together with love for our land. Naples certainly deserved this tribute as a recognition of its glorious past and its tradition of pole of attraction for excellent minds and inclusive culture.

This was not unexpected in Neapolitan history but determined by peculiar events, both human and geographical that, I believe, led to make this region of the world a favorable one to become a hub for philosophical and intellectual speculation. And science is, as we all know, fundamentally the result of intellectual and philosophical speculation, nothing else.

The same drive that attracted the divine Vergil to the Neapolitan epicurean school of Chiron, his master and philosophical mentor for masterpieces as the Georgics, led a young and enlightened German emperor in the early Middle Ages to promote Naples as the center of culture, founding the oldest University of Europe and therefore of the western world. The "Studium" established in Naples by Friedrich II Hoenstaufen, emperor and innovator, "*stupor mundi*" as he was called by most historians, was not technically speaking the first one of its kind. The University of Bologna preceded it of many years, but Naples University was the first public institution of a State, born for the political will of a Ruler whose project was that of creating a place where studies were possible without having people leave home, and constituting a center of attraction for scholarly minds. It was an operation of qualified touristic promotion, we might say today, where thegoal was the cultural growth of the place that would become in turn economical and social. At that time is ascribed the myth of the four founders, a Jew, an Arab, a Greek and a Latin, not real individuals but cultural influences concurring to the building of the *ars medica*, and the body of laws that regulate the teaching and the practice of medicine as stated in the "*Liber Constitutionum"* in A.D. 1231. Philosophical speculation and observation of the reality, theory and practice, *ratio et observatio* were the leading criteria for the development of scientific rationales and approaches.

Naples has therefore been since the far past the place where different culture met and merged, creating one of the first melting pots of peoples and ideas in history, favored by its geographical position at the center of the Mediterranean and by the efforts of enlightened rulers, like Friedrich II and, more recently, like The Bourbon kings. Starting with Charles III Bourbon, in fact, Naples became along with Vienna and Paris one of the best and most advanced courts in Europe, both for magnificence of arts and for scientific institutions. The first railroad in Italy between Naples and Portici was built in the year 1836, the first Italian scientific museum of mineralogy was established in the city in 1801 and the first volcanic observatory of the world was built on the slopes of Vesuvius in 1841. Naples was also the place where, in 1872 the eminent German biologist Anton Dhorn built the second laboratory of marine biology in Europe, which hosted, among many others, Ilia Metchnikoff, the second Nobel prize winner for medicine. In Naples took also place the seventh Meeting of the Italian Scientists in 1845, with the participation of 16 hundreds scientists, more than 8 hundred of whom from Naples and the south of Italy. Cultural and scientific growth called for economic growth.

Other times have not been so favorable however. Periods of glory alternating with periods of decline, mainly determined by political instability and short-sightedness have determined the cultural oblivion that has especially characterized the scientific life of the city. It looked as though Naples, in spite of sporadic and meritorious efforts operated at different levels by singular initiatives, substantially relied on its traditional and popular image of a place to visit for touristic reasons. An initiative of restoring the glorious role of the past was at this point needed by many people operating in the scientific field. This led to a dreamer such as Professor Zappacosta to enterprise this initiative of creating a novel cultural start that would bring here the best of the world scientists who would in turn attract again an international qualified audience. The project was also highly

philanthropic, since a great deal of attention was dedicated to the students coming from developing Countries who were actively encouraged to attend the courses by granting them bursaries also obtained by international benefactors like the Bill and Melinda Gates Foundation. This was a theme particularly dear to the founders, who envisioned the city to become once again a bridge between the western world and less fortunate areas of the planet that would benefit of this shared knowledge and culture. I recognize in those ideals the reason for my enthusiastic adhesion to Serafino's generous effort and I will be always grateful to him.

The legacy of the Ceppellini School, still vivid in our minds, can be summarized in the motto suggested by Professor Zappacosta "*non multa sed multum*" ("not many but much", i.e., "quality, not quantity"), that best represents the spirit of an institution that is progressively changing the perception of ourselves and of the role of the western culture in the world.

This perfectly fulfills the prophetic perspective of Serafino Zappacosta for a new era of Immunology and Immuno - Oncology in which a more humanistic and philosophical approach should prevail in research. May the Ceppellini School continue to be a beacon for decades to come.

AUTHOR CONTRIBUTIONS

AD is a co-founder of the School of Immunology.

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Serafino Zappacosta: An Enlightened Mentor and Educator

Ennio Carbone[1,2], Mario De Felice[3], Francesca Di Rosa[4], Ugo D'Oro[5], Silvia Fontana[3], Antonio La Cava[6], Michele Maio[7], Giuseppe Matarese[3,8], Luigi Racioppi[8,9], Giuseppina Ruggiero[10]* and Giuseppe Terrazzano[10,11]*

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With this article, the authors aim to honor the memory of Serafino Zappacosta, who had been their mentor during the early years of their career in science. The authors discuss how the combination of Serafino Zappacosta's extraordinary commitment to teaching and passion for science created a fostering educational environment that led to the creation of the "*Ruggero Ceppellini Advanced School of Immunology*." The review also illustrates how the research on the MHC and the inspirational scientific context in the Zappacosta's laboratory influenced the authors' early scientific interests, and subsequent professional work as immunologists.

Keywords: education, MHC, T cells, NK cells, immune response

Serafino Zappacosta, the founder of the "*Ruggero Ceppellini Advanced School of Immunology*" (Ceppellini School), epitomized the term "mentor." This term was first used by François Fénelon in the book "Les Aventures de Télémaques" to define an enlightened educator who is endowed with unprejudiced knowledge and wisdom (1). The name came after Mentor, the guardian and educator of Odysseus' son Telemachus who offered him encouragement and support for dealing with personal dilemmas while his father was away fighting in the Trojan War. As a mentor and professor of immunology at the University of Naples "Federico II," Serafino Zappacosta communicated science to his students and close collaborators as a fascinating tool to constantly pursue and advance knowledge, thus nourishing their innate human eagerness to learn. The authors of this article had the privilege to be former members of Zappacosta's laboratory, and wish to offer him this posthumous tribute.

Serafino Zappacosta was a highly cultivated scientist, whose interests spanned from the classics to arts (2). His open-minded vision led him to go beyond the traditional approach to didactics toward new models of education. He conceptualized the idea of an international advanced school of immunology because of his recognition of the quintessential importance in committing educational efforts to nurturing generations of young researchers from all over the world, promoting exchanges between western world and developing countries. The Zappacosta's school model embraced an open and transparent communication that was instrumental to expand the creative potential of independent-minded young investigators, to foster their critical and analytical interests, and to channel their energies into highly valuable scientific directions.

Serafino Zappacosta founded the Ceppellini School in 1991 together with Antonio Di Giacomo (Experimental Immunologist working at the Monaldi Hospital in Naples, Italy), Melchiorre Brai (Professor of Immunology at the University of Palermo, Italy), Giovan Battista Ferrara (Professor of Human Genetics at the Federico II University of Naples, Italy), Albert Nisonoff (Professor of Biology at Brandeis University, in Waltham, Massachusetts, USA), and Ciro Manzo (Head of Immunology Department at the Istituto Pascale in Naples, Italy). The choice of Naples as Ceppellini School headquarters was no accident. This city had experienced in 1799, during the Neapolitan Republic, an unsuccessful attempt to gain freedom from the constraints of a tyrannical monarchy, and to promote a new political organization. This attempt was brutally suppressed. The choice of Naples symbolically reflected the will to propel the freedom of scientific minds according to the School's motto "*non multa sed multum*" ("not many but much," i.e., quality rather than quantity) (3). Over the years, the Ceppellini School has been attracting and engaging large numbers of international young scientists to the field of immunology.

FOCUSING ON MHC MOLECULES

Serafino Zappacosta had a strong interest in the major histocompatibility complex (MHC) genes and proteins. He shared his fascination for the MHC with his laboratory members, with whom he investigated a variety of topics in the MHC field, including the regulation of HLA expression in tumor cells (4, 5), the association between HLA alleles and diseases in Southern Italy (6, 7), the influences of MHC class I (MHC-I) on tumor killing by NK cells (8), and the cytokine-mediated regulation of MHC-I expression (9). His laboratory also worked in collaboration with many international teams to study HLA polymorphisms, and participated in collaborative workshops on HLA typing, that included the International Histocompatibility Workshop in 1991 in Yokohama, Japan (10). This interest in MHC also led to the name of the Ceppellini School, after the immuno-geneticist Ruggero Ceppellini, who had greatly contributed with his pioneer work to the understanding of the genetic bases of HLA polymorphisms, and coined the term "haplotype" (11).

MHC POLYMORPHISM AND THE MEDITERRANEAN AREA

Before molecular genetics could rely on modern technology to collect a tremendous amount of information, many data about the genetic background of different human populations were based on the analysis of products of polymorphic loci including HLA. Analyzing the HLA system at the end of the '70s, Zappacosta, together with Mario De Felice, Michele Fiore and Giovan Battista Ferrara (12), found significant differences between people living in Northern Italy and the population of Campania (in Southern Italy). Significant similarities were noticed between Mediterranean and Middle Eastern populations and people from Campania, confirming that the genetic background of the Italian population is highly mixed. Furthermore, a peculiar association between HLA alleles and congenital adrenal hyperplasia was found by Serafino Zappacosta, Michele Maio, Mario De Felice and Rossella Valentino in the Sothern Italian population (6). These studies were performed at the time when Luigi Cavalli-Sforza investigated the selection of advantageous alleles in HLA locus and other polymorphic loci, whereby those findings served to illustrate migration patterns of human populations (13).

Further studies on MHC polymorphism were performed over the years by Giuseppina Ruggiero, Giuseppe Matarese, Giuseppe Terrazzano, and others in the Zappacosta's laboratory. They demonstrated a link between HLA alleles and susceptibility to autoimmune/infectious diseases in Southern Italy (7, 14). Other Zappacosta's team members, including Michele Maio, Luigi Del Vecchio, and Mario De Felice, documented the association between HLA-DR alleles and thyroid carcinoma (15). In the 80', Michele Maio, Luigi Del Vecchio, Giuseppina Ruggiero, Mario De Felice and others of the Zappacosta's laboratory investigated MHC-I expression as a prognostic factor in breast cancer (5). Antonio Pinto, Michele Maio and others showed that HLA-DR expression by myeloid leukemia cells was modulated by anti-neoplastic drugs (4).

MHC MOLECULES AND THE REGULATION OF NK CELL RESPONSE

In the late '80, Silvia Fontana, Luigi Racioppi and Ennio Carbone in Zappacosta's team investigated the link between retroviral infection and MHC-I expression by tumor cells, using virus-induced rat thyroid adenocarcinomas as an experimental model (16, 17). At the time, state-of-the-art techniques for these studies included tissue culture, microscopy, immunofluorescence, and cytofluorimetry, that had only become available a few years earlier. Silvia Fontana, Ennio Carbone, and others in the team showed that tumor transformation modulated MHC-I expression by rat tumor cells, and that rat Large Granular Lymphocytes (LGL) killed more effectively tumor cells having low MHC-I expression (8). These observations were puzzling at that time. In 1987—just a few years before these studies—, Bjorkman and colleagues had solved the HLA A2 crystallographic structure (18, 19), and a lot of attention was concentrated on the TCR/MHC-I molecular interaction, and its role in immunity. On the other hand, Ennio Carbone of the Zappacosta's team was fascinated by pioneering studies on the inhibitory signals provided by MHC-I to Natural Killer (NK) cells [the "missing self" hypothesis, formulated by Klas Karre in 1981 (20)]. Together with Antonio La Cava, Giuseppe Terrazzano and others, the Zappacosta group's contributions to the NK field spanned from MHC-I mediated regulation of NK cell cytotoxicity in rat tumor models (8, 21), to NK cell inhibition induced by soluble HLA-I (22), to new findings on the role of NK cells in human tumor immunosurveillance (23), NK/ dendritic cells cross-talk (24, 25), and CD1-mediated inhibition of NK cytotoxicity (26). Giuseppe Terrazzano in the group investigated the effects of IL-10 on MHC-I expression and on the antigen presenting machinery

(9), demonstrating a pathological role of gliadin in the NK cell/dendritic cells cross-talk (27). Sadly, this publication was the last one that included Serafino Zappacosta's co-authorship.

MHC MOLECULES AND THE REGULATION OF T CELL RESPONSE

In the early 90's, it was well-established that the function of MHC molecules was to bind and present antigenic peptides to T cells (28, 29). The molecular bases of this phenomenon had been largely resolved by several independent studies (30–35). However, looking out of this canonical box, it was possible to hypothesize that, in addition to binding TCR and CD4, MHC class II (MHC-II) molecules might also interact with other cell surface molecules, and in turn regulate the activation of immune cells. Within this context, Zappacosta's group aimed to identify non-canonical functions of HLA-II molecules (36–39). A large panel of monoclonal antibodies (mAbs) directed against different epitopes of HLA-II molecules provided by Soldano Ferrone (40) were instrumental for these studies, that were performed in *in vitro* models of polyclonal T-cell proliferation, induced by either phytohemoagglutinin (PHA) or anti-CD3 mAb (41, 42). Although the presence of antigen presenting cells (APC) was required to achieve full T-cell activation, HLA-II antigen presenting function was largely dispensable in these models, thus offering a unique opportunity to evaluate non-canonical functions of HLA-II molecules.

Giuseppina Ruggiero and Luigi Racioppi in Zappacosta's team, in collaboration with Ciro Manzo, initiated these pioneer studies on HLA-II molecules, and demonstrated that the incubation of autologous monocyte-derived macrophages with mAbs directed against non-polymorphic determinants of HLA-II molecules exerted a remarkable inhibitory effect on T cell activation (37, 38, 43). This result suggested that MHC-II molecules expressed on the APC could interact not only with the TCR and CD4, but also with additional ligand(s)—at the time unknown—, expressed on the T cell surface. Interestingly, in 1996 one of these hypothetical ligand was identified by Huard et al. (44), who demonstrated the ability of CD223 (aka LAG-3) to bind MHC-II molecules. In the last two decades, the relevance of LAG-3/MHC-II signaling has been confirmed by several independent studies, being this molecular interaction involved in a variety of immuno-regulatory circuits (45).

D'Oro and Di Rosa from the Zappacosta's team further explored non-canonical functions of MHC-II molecules expressed by activated human T cells. These studies were based on the general hypothesis that HLA-II molecules might transduce intracellular signals, and in turn finely tune T cell response to antigen(s) and cytokines. The results confirmed this possibility, showing that cross-linking of HLA-II on activated T cells was sufficient to induce inositol triphosphates (IP3) accumulation, protein kinase C (PKC) activation, and, ultimately, enhanced T cell proliferation (10, 39). Of note, the ability of MHC-II molecules to transduce intracellular signals has also been recognized in B cells (46–48), and more recently a cell-intrinsic contribution of MHC-II expression has been shown in the B cell development in the bone marrow (49).

As a note, the findings by Zappacosta's group on non-canonical functions of the HLA molecules have relevant, and still largely unexplored, implications in the regulation of the human immune response. For example, high expression of LAG-3 by T regulatory (Treg) cells suggests that LAG-3/MHC-II complexes might play an important role in the bi-directional signaling triggered by Treg/ T effector cell interactions (50, 51). In this sense, an increasing number of studies has pointed to LAG-3/MHC-II interaction as an attractive druggable target to treat autoimmune diseases, stimulate anti-cancer immune response (52), and revert resistance to anti-PD-1 immunotherapy (53).

MHC AND BEYOND

Giuseppe Matarese devoted his experimental efforts on the innovative hypothesis that nutrient-energy-sensing pathways might represent a powerful tool to control immunological self-tolerance. He showed that leptin, a hormone critically involved in energy balance and body weight regulation, acts as a strong immune-modulator, that influences the susceptibility to infection and autoimmunity (54, 55). Leptin levels inversely correlated with regulatory T cell number in multiple sclerosis patients (56), and a direct link between leptin and regulatory T cell anergy was established (57). This observation, that was further developed in subsequent studies performed by Giuseppe Matarese and his group, in collaboration with Antonio La Cava, was the result of frequent, endless, unforgettable evening lab discussions with Serafino Zappacosta.

After training with Serafino Zappacosta with a focus on the immune-modulating properties of MHC molecules, the team members subsequently developed new hypotheses and investigations in diverse directions, ranging from the study of the fundamental mechanisms of immune regulation and immunological memory, to autoimmunity, cancer immunotherapy and vaccinology (58–66). Serafino Zappacosta kept to enthusiastically follow the progress of the past members of his team after they left to start their independent careers. Many of them remained involved over the years in the Ceppellini School activities, either as faculty members or as components of the board of directors, maintaining the School as an arena of continuous scientific education and dynamic discussion.

CONCLUSIONS

The review summarizes the legacy left by Serafino Zappacosta to his collaborators who, albeit with different individual perspectives and at a different degree, continued to work on the MHC, looking at these molecules as a window of opportunity to comprehend the complexity of the immune response, rather than merely looking at them as antigen presenting molecules.

After the death of Serafino Zappacosta in 2006, Silvia Fontana became the President of the Ceppellini School, and Ennio Carbone, Giuseppe Matarese, Francesca Di Rosa, Giuseppina Ruggiero, Giuseppe Terrazzano and other previous collaborators of the Zappacosta's team continued to organize advanced international immunology courses, together with the

long-standing Serafino Zappacosta's collaborators and friends Antonio Di Giacomo, who co-founded the School in 1991 (3), Elizabeth Simpson, who organized the first Ceppellini School Course in 1992 on bone marrow transplantation (67), and the newly recruited Ceppellini School Scientific Director Stefan Kaufmann (68). A new type of event, the Serafino Zappacosta Memorial Conferences, was initiated in 2007. Since 2010, this event has been held in the newly inaugurated "Serafino Zappacosta" Auditorium of the Federico II University of Naples. All these activities were made possible by the excellent work of the Ceppellini School Scientific Secretary Tricia Reynolds.

To conclude, the continuation of the activities of the Ceppellini School not only allows an unceasing engagement of new young bright minds to the fascinating field of immunology, but also keeps alive Serafino Zappacosta's dream that intellectual freedom can be shared without boundaries for the benefit of younger generations.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DEDICATION

This article is dedicated to the memory of Luigi Del Vecchio, past member of Zappacosta's team and full professor of Clinical Biochemistry and Molecular Biology, Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II," Naples, Italy at the time of his premature death in 2018.

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Conflict of Interest: UD is an employee of the GSK group of companies and holds restricted shares of the GSK group of companies.

The remaining 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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Ruggero Ceppellini: A Perspective on His Contributions to Genetics and Immunology

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Ruggero Ceppellini, who died at the age of 71 in 1988, was one of the most stimulating and original human geneticists of his generation (1). Ceppellini's outstanding contributions to the genetics of the human blood groups, immunoglobulin allotypes and the HLA system epitomize the study of immunogenetics. By using his considerable skills and insights to unravel the interpretation of the serological data, he made significant contributions to immunology. He is remembered especially for his incisive contributions to the development of the genetics of the HLA system and its nomenclature, including, in particular, his introduction of the term "haplotype," now widely used by geneticists throughout the world, most of whom are unlikely to be aware of its origins.

Keywords: Ceppellini, HLA, MLC, haplotype, transplantation

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EARLY BLOOD GROUP DISCOVERIES

Born during the first world war, Ceppellini was caught up by military service in the second world war and so could not finish his medical studies until after the war had ended. During his service as a sergeant in World War II, Ceppellini was captured by the British and taken as a prisoner of war in Palestine, where the charismatic physician and human geneticist, Chaim Sheba, took him on as a medical orderly because of his medical background. Many years later, when Ceppellini was attending a human genetics meeting in Israel, Chaim Sheba greeted him as "Sergeant Ceppellini." Perhaps it was that brush with genetics that stimulated his interest in the field and led to his appointment, through the influence of Luca Cavalli-Sforza, to a position in the Istituto Sieroterapico Milanese, a blood bank associated with the University of Milan. Cavalli–Sforza, although Ceppellini's junior by 5 years, was already becoming established as a geneticist and was a major influence on Ceppellini's future career.

In 1954, Ceppellini was invited to work in the Institute for the Study of Human Variation at Columbia University in New York, where he came under the influence of L. C. Dunn and made his first significant contribution to immunogenetics. He showed, through a careful family and population based study, that the Rh variant D^u was actually due to a reduced expression of D when associated in heterozygotes with the combination Cde (2).

Shortly after his return to Italy in 1959, Ceppellini made his second outstanding contribution to the blood grouping field. This was his interpretation of the Lewis b phenotype as an interaction between the secretor and Lewis genes, and his interpretation of the ABO and Lewis blood groups in terms of a form of metabolic sequence involving successive additions of sugars. His model, based entirely on a genetic interpretation of the data, showed remarkable insight and was abundantly confirmed by the studies by Morgan, Watkins, Kabat, and others of the oligosaccharide structures of these blood group determinants and the eventual identification of the two fucosyltransferase genes (3, 4).

MALARIA, THALASSAEMIA AND THE IMMUNOGLOBULIN (GM) ALLOTYPES

In the early 1950s, Ceppellini had started a systematic study in Sardinia to correlate the distribution of thalassaemia, which was common there in the low lying villages, with the distribution of malaria that had been established in Sardinia by an extensive study of school children. Through this, he provided some of the first clear evidence of the correlation between thalassaemia and resistance to malaria, showing that its frequency was highest in those areas where the incidence of malaria had been greatest (5). His work there stimulated a long and continuing tradition of studies in human genetics in Sardinia, carried forward especially by his close friend and colleague Marcello Siniscalco.

My initial contact with Ruggero Ceppellini was established through Luca Cavalli-Sforza, in the early 1960s, because of my interest in the studies of thalassaemia and malaria as a model of natural selection in human populations. I, and my population genetics colleagues at Stanford University, invited him as a population geneticist to discuss this work. In characteristic manner, just before he was due to arrive, we received a telegram saying that unfortunately, after all, he was not able to come.

Stimulated by his contact with Henry Kunkel during his time in New York, Ceppellini took up the study of the Gm types. Kunkel was a pioneer of the study of the single immunoglobulins produced by myelomas, while Grubb had shown in 1956 (6) that there were inherited serologically detectable differences in the immunoglobulins, which were called Gm allotypes, G for immunoglobulin G and m for marker. By the early 1960s, the basic two chain structure of the immunoglobulins and the distinction between constant and variable regions had been elucidated, so that it became clear that these allotypes were inherited variations in the IgG heavy chain constant regions.

In a major comprehensive and inciteful review of the Gm allotypes, published in Italian in the proceedings of the 1966 meeting of the Italian Genetics Association (7), Ceppellini provided what was at that time the clearest interpretation of the Gm types as a complex genetic system. This was in some ways analogous to the Rhesus blood group system as interpreted by R. A. Fisher, on which he had published earlier with L. C. Dunn. His interpretation was in terms of "haplotypes" (an expression first used in this paper), which determined different combinations of Gm types, and their varying frequencies in different populations. He appreciated the possibility of the creation of new haplotypes by recombination between existing haplotypes and extended some of the formulae, which I had developed for the analysis of two locus-linked systems, to the estimation of haplotype frequencies. This notable paper is hardly ever quoted because of its publication in Italian in a more or less inaccessible journal. It is also, to my mind, odd that, as far as I am aware, this is his only publication on the Gm types. Perhaps that is because it was at this time that he started on his major interest in what became the HLA system.

HLA, HAPLOTYPES, CELLULAR ASSAYS, AND MONOCLONAL ANTIBODIES

Early White Cell Agglutination Serology

Jean Dausset pioneered the testing of sera from multiply transfused patients against white blood cells from arbitrary volunteer donors using an agglutination reaction. His aim was to establish whether these reactions could be interpreted to define inherited blood group like determinants on white rather than red blood cells. The initial results, not surprisingly in retrospect given the now known complexity of the HLA system, were very confusing.

Ceppellini suggested to Dausset in 1956 that he should compare the reactions on white cells from pairs of identical (monozygous or MZ) twins with those on pairs of non-identical (dizygous or DZ) twins. Then, if the agglutination reactions reflected inherited determinants, all the reactions should, subject to experimental error, be the same on each of the MZ twin pairs, while this would not necessarily be the case for the DZ twins. Dausset and Brecy published a short note in Nature in 1957 (8) confirming this prediction, following which, Dausset described in 1958 a putative first antigen, which he called MAC (9), and for which he shared the Nobel Prize in 1980.

The serology of the white blood cell antigens did not, however, progress any further until Jon van Rood and Rose Payne, independently in 1958, showed that sera from multiparous women contained antibodies against the white cells of their offspring that could be detected by agglutination assays. These were produced by fetal-maternal stimulation, just as in the case of the Rh blood groups and, being limited to the difference between mother and father, were much less complex than sera obtained from multiply transfused individuals. By the early to mid 1960s, Ceppellini had joined the initial group of workers in this field. In the first of the International Histocompatibility Testing workshops, organized by Bernard Amos in 1964, Ceppellini played a major role in analyzing the data and promoting the need for improved reproducibility of the testing techniques as well as for inter-laboratory comparisons of results. [For details of the early history of the HLA field, as described by its pioneers, see (10)].

As a geneticist, Ceppellini appreciated the importance of family studies, and so organized, with his colleagues, the third International Histocompatibility Testing Workshop in Turin in 1967 around the theme of a family study. While by that time it was clear that there were at least two, probably closely linked, loci for the white blood cell determinants being described, this had not been definitively established by family studies. He provided the families and we came with our sera and different technologies to test white blood cells from his family members. Ceppellini expressed to the press his amusement at seeing an erstwhile mathematician sitting at the bench looking down a microscope. The aim of the collaborative on site study was to see whether all the types being defined by the different participating laboratories were inherited together—and they were!

This, then, was the first clear-cut establishment of the HLA system as a set of closely linked genes inherited together in

a way that was analogous to the Rhesus blood groups and immunoglobulin Gm allotypes.

Haplotype

It was notably at this workshop in 1967 that Ceppellini first really introduced the term "haplotype," though he had used it the previous year in the Italian Gm allotype review, as already mentioned. His description was as follows:

"If a new term can be introduced without increasing confusion, it is suggested to substitute phenogroup with haplotype (haploid, from απλόος, single); in fact, the name should convey the concept that the haplotype is not an observed phene and corresponds to the product of a single gene dose." My interpretation of this, as given in Cavalli-Sforza and Bodmer [1971, though written in 1969, (11)] was: "**haplotype** (from haploid genotype) for the combination of genetic determinants that leads to a set of antigenic specificities which is controlled by one chromosome and so inherited in coupling."

The term was originally conceived in the context of a tightly linked cluster of alleles in strong linkage disequilibrium and before the advent of DNA based technology with its almost unlimited number of polymorphisms. However, it can clearly be generalized to refer to the set of variants to be found on any given stretch of DNA on one of the two homologous chromosomes in an individual. That DNA could extend from as little as a single exon, within which there is more than one variable position, to a whole chromosome. The concept therefore becomes vague unless it is related to a defined stretch of DNA.

Skin Graft Survival and Blood Group Incompatibility

There was an obvious interest in establishing whether the newly identified white blood cell determinants were histocompatibility antigens in the sense of being responsible for graft rejection when not matched. Early data had suggested this was the case. Ceppellini and colleagues exchanged grafts between sibs, parents and offspring, and unrelated individuals in a systematic design. Through a collaboration with van Rood, they showed that skin graft survival times were longer when individuals were matched for the groups defined by van Rood's leukocyte agglutination assay than when they were not (12, 13).

Mixed Lymphocyte Culture (MLC) Reaction

The Mixed Lymphocyte Culture (MLC) reaction, in which lymphocytes from different individuals when cultured together stimulate a mutual mitotic blast response while lymphocytes from the same individual do not, was discovered by Bach, and independently by Bain, in 1964. This creation of a sort of *in vitro* model for homotransplantation at the cellular level intrigued Ceppellini sufficiently to lead him to invite Fritz Bach to his laboratory to demonstrate his test. Following this, Ceppellini's group developed a "one way" MLC, in which only one of the pair of lymphocytes in a co-culture was able to respond. They then showed that some sera containing anti HLA antibodies were able to block the MLC reaction (14, 15). Ceppellini's group just missed making the important observation made by Bach and Amos (16) that MLC reactions associated precisely in families with the then serologically defined leukocyte antigens.

This discovery, however, was surely stimulated by Ceppellini's discussion of genetics with Fritz Bach. Van Rood's group was the first to define serological reactions correlating with MLC reactivities, using a rather cumbersome technique involving inhibition of MLC based on Ceppellini's discovery. This laid the foundation for the discovery of the HLA – DR and other Class II determinants using simpler B cell specific serological techniques with the same sources of sera that were used to define the HLA -A, B and C antigens. It is these anti-HLA-DR and related antibodies that explain the MLC inhibition that Ceppellini's group had first observed (see 10 for further details).

Monoclonal Antibodies, Disease Association, and Nomenclature

Ceppellini became one of the first members of the Basel Institute of Immunology in 1970 and so was amongst the first to realize the importance of monoclonal antibodies, in particular in their application to the HLA system. It was one of his antibodies, produced with Massimo Trucco, that was first used in an International HLA Workshop in 1980. Ceppellini embraced the concept that monoclonal antibodies were the analytic tools of the future and moved from the Basel Institute to Roche as a scientist with the specific objective of producing human monoclonal antibodies. He was, as I recall, quite disappointed in the apparent lack of interest of the Roche pharmaceutical company in the work produced by the outstanding institute that they had created and supported.

In the late 1960s, Ceppellini was, not surprisingly given his background of work on thalassaemia and malaria in Sardinia, one of the first to promote the idea of looking for associations between HLA variants and diseases. It was a follow up of his work in Sardinia that first provided substantial evidence for a role for HLA in malaria resistance (17).

Ceppellini had remarkable insight not only into the genetics and biology of the HLA system but also into the quantitative aspects of its interpretation, as evidenced, for example, by his analysis of the Gm haplotypes. This brought us together in a joint publication from the 1970 International Histocompatibility Testing Workshop on the formal theory of testing the fit of two- and three-locus models of the serological data on HLA at that time and on the analysis of segregation patterns in families (18). My wife, Julia, and I remembered vividly his stay with us in California in 1970 where we finished writing the paper. He had broken his arm skiing, his favorite sport, and he was as lively as ever, but nevertheless, a broken arm did to some extent inhibit his speech! Ceppellini had, much earlier, made a significant contribution to what became a classical method for estimating gene frequencies in a random mating population using iterative gene counting (19).

Ruggero Ceppellini was one of the only professionally trained geneticists among the early workers in the HLA field, apart from myself. Through this we developed a rapport and friendship over a period of more than 20 years.

His clear thinking and forceful contributions to discussions of the WHO International Nomenclature Committee meetings helped enormously in the development of a rational HLA nomenclature based on a proper understanding of the genetics. Ceppellini shares with me and Jon Van Rood the responsibility for the HLA-DR nomenclature and so, eventually, DP and DQ.

Conclusion

Ruggero Ceppellini unfortunately suffered from periods of depression during the 1970s. His manic periods were easily identified by long and stimulating phone calls in his characteristically deep-throated Italian accent, which would come at any time of the day or night. Indeed, the last communication I had from him, 10 days before he died, was an offer of his lung ascites to culture his tumor cells and a request for references on the genetics of lung cancer.

Ceppellini was undoubtedly one of the most charismatic and original thinkers in the fields of human genetics and immunology in the second half of the 20th century. He is, however, in my view, not sufficiently appreciated for his many scientific contributions. It is gratifying, therefore, to see his memory sustained by the European Federation of Immunogenetics (EFI) annual Ceppellini award lecture, first given by Jon van Rood very shortly after his death, and the stimulating Ceppellini advanced courses in immunology organized by the "Scuola Superiore d' Immunologia Ruggero Ceppellini".

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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Bone Marrow Transplantation 1957-2019

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Clinical bone marrow transplantation started in 1957 at a time when remarkably little was known about hematopoietic stems cells, immune responses to transplants or the identity of transplant antigens. This review will delineate the substantial increase in knowledge about these three areas gained between then and 1992 when the Ceppellini School course on Bone Marrow Transplantation was held, along with the progress made in clinical application, as well as the stumbling blocks that remained to be overcome by further research to advance knowledge. It will outline the significant progress made between 1992 and the present year, 2019, and the remaining problems.

Keywords: transplantation, histocompatibility, graft-vs.-host, graft-vs.-tumour, immunosuppression

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INTRODUCTION

The Scuola Superiore d'Immunologia Ruggero Ceppellini (Ceppellini School) was founded in 1991 in Naples by Professor Serafino Zappacosta, to honor the memory and achievements of Professor Ruggero Ceppellini, a giant in the field of HLA genetics, who led an approach to addressing complex scientific questions through national and international collaboration (**Figure 1**). The Ceppellini school takes this forward by promoting contact and collaboration through residential post-graduate level courses led by international faculty for early career basic scientists and clinicians from advanced and developing regions and countries. It has accelerated Immunology education and influenced the evolution of other international schools of immunology. This issue of *Frontiers in Immunology* is a celebration of the achievements of the school and a tribute to Professor Serafino Zappacosta, Professor of Immunology at the "Federico II" University of Naples, who aimed to create in Southern Italy a pole of attraction for those pursuing immunological studies, and to promote interaction among the scientific and medical communities at the national and international level.

REVIEW OF THE BONE MARROW TRANSPLANTATION COURSE

In 1992 Bone Marrow Transplantation was the subject of the inaugural course of the Ceppellini School. This topic brought into focus both genetics and immunology, the areas to which Ceppellini's research on hematological disorders and the human major histocompatibility complex, HLA, was pivotal.

This review of bone marrow/hematological stem cell transplantation will focus on how contributions to the 1992 Ceppellini School course on Bone Marrow Transplantation provide a mid-way marker point in the six decades following 1957 when Donnall Thomas first reported on six patients given bone marrow transplants to restore hemopoiesis following ablation by radiation or drug toxicity (1). He was encouraged by Peter Medawar's 1953 report (2) that immunological rejection of skin grafts exchanged between non-genetically identical mice could be abrogated by induction of transplantation tolerance and by Loutit's work showing restoration of hemopoiesis in irradiated mice given spleen cells from the same inbred strain, but not from a different strain (3).

FIGURE 1 | Naples, where Professor Serafino Zappacosta founded the Scuola Superiore d'Immunologia Ruggero Ceppellini in 1991. Image: Vera Maone, used with permission.

At that time, in the 1950s, there was limited knowledge of the genetics of transplant antigens and the immune responses to them, and all of those first patients died, although transient chimerism was recorded. In 2018, 60 years later, hundreds of thousands of hemopoietic transplants have been carried out, using a variety of sources for stem and precursor cells and an array of pre-conditioning treatments to facilitate graft acceptance in patients. While many recipients survived, cured of hematological malignancies or hematological diseases that would otherwise have killed them, others suffered serious side effects of which graft-vs.-host disease (GVHD) has been the most challenging. This uncomfortable "fact of life" has limited the more widespread use of hemopoietic transplants to treat other conditions that might benefit from "resetting the immune system," such as autoimmunity and rejection of therapeutic organ transplants.

Advances in pharmacology and the development of less toxic preconditioning regimes have made a series of stepwise improvements, both in graft acceptance and reducing GVHD incidence and severity. These have been built on advances in genetics, particularly with respect to delineation of the major histocompatibility complex (MHC), HLA in humans, along with homologs in species used in preclinical research, mice (H2), dogs (DLA), and non-human primates (SLA). In parallel, increasing knowledge of the immune system has provided insight into factors regulating the quality and quantity of immune responses, and has triggered the development of a range of biologically active pharmaceuticals aimed at controlling over- or under-effective responses in patients. The enrolment of patients into controlled clinical trials is the ultimate way to test safety and efficacy of new treatments: this is now widely embraced.

Our speakers in 1992 included those working on hematopoietic stem cells (Nydia Testa, Maria Grazia Roncarolo, Peter Hoogerbrugge) on identification of major and minor histocompatibility antigens (Robert Lechler, Elizabeth Simpson, Giovanni Ferrara), on immune responses to transplants (Herman Waldmann, Manlio Ferrarini, Yair Reisner) and on treating patients with hematopoietic disorders with HSC transplants (Jill Hows, Andrea Bacigalupo, Bruno Rotoli, Andrea Velardi, Guido Lucarelli).

IMMUNE RESPONSES TO TRANSPLANTS

Hemopoietic stem cell transplantation (HSCT) is the forerunner of both cell and gene therapies, which depend on slipping potentially foreign components past homeostatic controls limiting cell numbers and immune responses fine-tuned by evolutionary selection for protection against pathogens. The numbers of cells comprising some tissues can be reduced by irradiation and/or cytotoxic drugs to provide "space" for the introduced cell population to settle and proliferate. The appropriate dose of space-inducing treatment depends on the tissue and on whether total replacement or chimerism is required for therapeutic effect.

However, the immune response remains a formidable barrier, comprised of a moving army of variously armed host cells along with cell-bound and shed molecules, such as antibodies, receptors and cytokines, orchestrated by a complex activatory and inhibitory pathways. For hemopoietic transplants the situation is further complicated by potential two way reactions between recipient and donor: rejection of donor cells is the host-vs.-graft (HVG) response, whereas attack of the host by cells in the donor innoculum is the graft-vs.-host (GVH) response. Graft-vs.-host disease (GVHD) occurs when normal host tissues are attacked, but when this is focused on host tumor cells, the terms graft-vs.-leukemia (GVL) or graft-vs.-tumor (GVT) are used. Separating GVHD from GVL/T has proved difficult. Though most of the target antigens are shared, in principle there could be a set of non-shared tumor antigens. Unfortunately, which patients will develop GvHD and/or GVL cannot yet be accurately predicted because the molecular targets have not been sufficiently identified. The extraordinary diversity of target antigens is amplified by HLA polymorphism as well as that of minor histocompatibility antigens and tumor antigens arising from serial mutation.

Peter Medawar demonstrated that rejection of skin grafts exchanged between genetically dissimilar rabbits or mice showed specificity and memory (4)—hallmarks previously ascribed to antibody responses against pathogens. Mitchison subsequently transferred skin graft rejection with lymphocytes, but not serum, i.e., it was cell and not antibody mediated (5). On the basis that immune responses evolved to discriminate between self and non-self, Medawar designed experiments in which cells from one inbred mouse strain were introduced to immune-incompetent pre-natal or neonatal mice of another strain to induce recognition of them as "self" during development. When skin grafted as adults, most of the injected mice showed prolonged acceptance of test grafts. These experiments were replicated in other mammalian species and in birds (2, 6). Thus, the possibility of inducing transplantation tolerance existed, giving encouragement to both hematologists like Donnall

Thomas and surgeons like Joseph Murray who performed the first kidney transplants in humans.

Inducing tolerance in adult animals, either humans or experimental species, proved more difficult. Making recipients immunoincompetent using irradiation and/or cytotoxic drugs abrogates HVG but can lead to collateral damage to host tissues, and if immunocompetent lymphocytes of donor origin are included in the donor graft, they can cause GVHD. The morbidity and mortality figures during the early years of clinical BMT were daunting but they sparked extensive and focused preclinical experiments in outbred dogs (by Thomas' group) leading to step-wise improvements in the clinical protocols used, including reduced levels of irradiation and the development of less toxic drugs for pre-treatment of recipients. Pretreatment of donor cells was also trialed including removal of contaminating lymphocytes from bone marrow and use of alternative sources, such as mobilized stem cells isolated from peripheral blood or cord blood as a source.

In the 1960s and 1970s basic research studies had probed the composition of lymphocyte populations, leading to the understanding of the different functions of thymus derived (T cells) and bone marrow (B cells) lymphocytes and of T cell subsets, Th helper, and Tc cytotoxic cells. The development of monoclonal antibodies by Milstein and Kohler in 1975 (7) led to the isolation of highly specific reagents for identifying and separating cell types on the basis of cell surface molecules. Separation of cells with characteristic markers using the fluorescence activated cell sorter developed by Len Herzenberg in the 1970s and 1980s (8) was crucial to defining the phenotype and function of hemopoietic and lymphopoietic cell subsets.

Isolation from mouse bone marrow of selected populations containing a high proportion of haemopoietic stem cells (HSC) that could repopulate all lineages was shown by Weissman in the late 1980s and early 1990s (9). However, monoclonal antibodies defining the homologous population in humans have been more difficult to develop for approved clinical use. Even low levels of contaminating T lymphocytes in partially purified sources of hematopoietic stem cells can cause GVHD. If T cells are completely removed leukemic relapse is more likely, although that risk can be significantly reduced by donor lymphocyte infusion (DLI) following T cell depleted hematopoietic stem cell transplantation (10).

The molecular identification of the targets of transplant rejection (histocompatibility antigens) has played a central role in all clinical transplantation, but hematopoietic cell reconstitution presents the greatest challenges because of GVH, but also the greatest rewards, with the development of GVL/GVT effectors.

At the 1992 Ceppellini School BMT course the speakers outlined the key findings underlying the development of the field and presented them for discussion along with recent advances. It was clear that the problem of GVHD remained serious, with questions about identifying the best tolerable genetic match between donor and recipient, and how to minimize and treat this complication. Since 1992 then there have been substantial advances in knowledge of stem cell biology, the genetics of histocompatibility antigens and of the cells, molecules and regulatory circuits of the immune system, permitting further stepwise improvements, but now, having looked at the immune response, let us consider how transplantation antigen genes and their products were originally identified, especially as key research, including links with a range of human diseases, was carried out in Italy.

GENETICS, MOLECULAR IDENTITY AND FUNCTION OF TRANSPLANTATION ANTIGENS, MAJOR AND MINOR

George Snell took a systematic genetic approach to enumerating and mapping loci responsible for graft rejection with experiments transplanting skin and tumor grafts between inbred mouse strains, their F1 hybrids and backcross progeny. These were carried out at the Jackson Laboratory in the late 1930s before DNA, genes or chromosomes had been identified as units of inheritance. Instead, co-inheritance of traits mapped them into so-called "linkage groups." Snell numbered his histocompatibility loci, H1, H2, H3, etc., according to their apparent strength, with H2 the strongest, eliciting the fastest graft rejection. It was named the major histocompatibility (H) locus, with the others designated minor H loci. The agglutinating antibodies developed by Peter Gorer following immunization of different mouse strains were found to detect alleles of H2. Snell's 1948 paper (11) summarizes findings defining not only the major but also a number of minor H loci, of which more were found by Snell and his collaborators Bailey and Taylor who isolated and mapped each H locus in congenic and recombinant inbred mouse strains. However, while MHC antigens are highly polymorphic, their minor H counterparts are not. They are either di-allelic or characterized by one expressed and one non-expressed allele.

Ceppellini played a key role within the international consortium (12) in discovering the human homologs of H2 antigens, the human leukocyte antigens (HLA) named from their expression on human peripheral blood lymphocytes, to which agglutinating antibodies from multiparous women were found, directed against paternal alloantigens. Ceppellini's genetic studies in the 1950s on hemoglobinopathies, linking resistance to malaria and thalassemia, was followed by his immunogenetic research on red blood cell antigens, leading him to recommend to Dausset, then working on the elusive human leukocyte antigens using poorly reproducible agglutinating assays, the study of identical twins, whose reactions should be the same if the antigens were genetically controlled. The power of a genetic approach was used by Ceppellini when he HLA typed large families, identifying siblings inheriting the same parental HLA alleles, those with different alleles, and those with one shared allele. He then exchanged skin grafts between them and discovered while the most rapid rejection took place when no HLA alleles were shared, that for even completely HLA matched pairs graft rejection was only delayed by a week or 2 (13). These findings, in parallel with Snell's on mice, were evidence that minor H antigens also existed in humans. Confirmation of this comes from clinical bone marrow transplantation: HLA matched sibling recipients can still suffer life-threatening GVHD directed against minor H allo antigens.

A breakthrough in interpreting the human HLA antibody data accumulated during the international histocompatibility workshops came when viewed as a complex of linked loci, each with a number of alleles, rather than as a single locus. This arrangement had already been observed for the mouse MHC, H2, to consist of two linked polymorphic loci (named H2K and H2D), encoding cell surface molecules expressed on all lymphocytes in peripheral blood (PBL). Other mouse anti-H2 antibodies were found that reacted with B but not T lymphocytes. These were directed against the products of loci within H2 that distinguished alleles associated with the ability to respond to certain haptenated proteins, i.e., those were "Immune Response" (Ir) gene controlled. The Ir genes mapped between H2K and H2D of the mouse MHC complex on chromosome 17 and were named H2A and H2E. H2 studies were easier than those on HLA because of inbred mice, including congenic strains with selected alleles of H2 backcrossed onto a standard strain. Intercrossing could then be carried out to create H2 recombinants, allowing the study of individual loci. In outbred populations, such as humans HLA recombination occurs at a low frequency depending on the chromosomal distance between loci—HLA genes are closely clustered on human chromosome 6.

To resolve a nomenclature clash that had occurred between different laboratories working on human MHC, the first human HLA loci were named HLA-A and -B, and between them a third locus, HLA-C was mapped. The human homologs of mouse Ir genes mapped them just outside (centromeric) the A/B/C region and were named DR, DQ, and DP. Their cell surface molecules, like Ir genes of mice, are not expressed on all somatic cells but on human PBL they were detected on both B cells and activated T cells. Mouse Ir genes and their human homologs were designated MHC class II, to distinguish them from H2K and D, and HLA A, B, and C, that were known as MHC class I.

Understanding the functional homologies of the mouse and human MHC relied on the development of *in vitro* assays of T cell function. Proliferation assays developed by Fritz Bach identified MHC class II alleles as stimulatory in one-way mixed lymphocyte cultures (MLC) between two mis-matched individuals where cells from one were irradiated to prevent them proliferating (14). In parallel studies in other laboratories cytotoxic T cells (Tc) were developed in MLC, initially in mice (15). Tc effector cells in these cultures were directed against MHC class I antigens, while helper T cells, Th, specific for MHC class II were required for optimal responses (16). The same is true for both species.

In vitro cultures of lymphocytes were also used to examine the fine specificity of cytotoxic T cells against viral epitopes and minor (H) histocompatibility antigens. The paradigm changing paper on this was published in 1974 by Zinkernagel and Doherty, who showed that *in vitro* re-stimulated spleen cells from mice immune to lymphocytic choriomeningitis virus (LCMV) generated cytotoxic T cells that recognized virus only in association with self MHC, i.e., they were "MHC restricted." The same was found to be true for mouse and human cytotoxic T cells specific for the male specific minor H antigen, HY (17, 18) and all other minor H antigens. Although the manner in which T cells recognized both self MHC and a foreign antigen was not understood until the crystal structure of HLA-A2 was solved in 1987, showing a peptide fragment of viral or other origin held in the peptide binding groove of the MHC molecule (19). The generation of minor H specific T cells and clones provided key reagents for mapping and cloning the corresponding genes and identifying their MHC binding peptides. This is also true for a tumor specific antigens (TSA): Thierry Boon led investigation of these by generating TSA specific T cell clones (20), and used these to expression clone a range of melanoma and other tumor antigen genes and explored use of them to immunize patients. This approach also informs treatment of leukemia patients, given HSC.

The *in vitro* T cell correlates of HVG and GVH immune responses against minor H antigens are MHC class II restricted Th helper cells and class I restricted Tc cytotoxic effectors. Unlike *in vitro* responses to mismatched polymorphic MHC antigens that develop in primary cultures, those to minor H antigens require previous *in vivo* exposure to antigen to increase the precursor frequency of T cells specific for the minor H antigen(s). Two MHC matched individuals in an outbred population will differ at many minor H loci, including HY in the case of brother/sister pairs. Immunodominance of the response against one or more minor H antigen occurs. An *in vivo* manifestation of this is stronger GVHD in male recipients of HLA matched female hematopoietic transplants than in female recipients, since HY is immunodominant to some other minor H antigens. However, the strength of each is also a function of the MHC restricting molecule, since it is the combination that determines immunogenicity.

The 1992 Ceppellini course on BMT included presentation and discussion of the research leading up to the discovery of both MHC and minor H antigens in humans and mice. This included methods then currently in use for HLA typing, and how MHC restriction prevailed for the recognition by T cells of all non-MHC antigens, whether minor H, viral and other.

Responses against multiple minor H antigens are strong and can be life threatening in GVHD. In contrast, if they could be separated into their components specificities they could be therapeutic, particularly for selecting those directed against minor H antigens preferentially expressed on tumor cells. An opportunity to create this situation occurs in leukemia patients given HSC transplants. Providing that expression of a minor H antigen is hematopoietic cell specific, cytotoxic T cells directed against the recipient allele of that antigen will target both leukemic cells and residual recipient hematopoietic cells but not those of repopulating donor origin. Such curative T cells would remove leukemic cells without the side effect of damaging other recipient tissues.

The treatment outlined above would require identification of minor H antigens expressed only on hematopoietic cells, for which there are some candidates, together with the isolation and expansion of effector T clones that can be approved for clinical use. Those of donor stem cell origin would be ideal, as they should persist long term in the recipient and mitigate against leukemic relapse. Current research on effector cells transduced with CAR-based or T cell receptor (TCR)-based constructs is relevant to this.

SOURCES OF HEMATOLOGICAL STEM CELLS (HSC)

HLA typing was recognized early as crucial for allogeneic HSC transplantation, as HLA mismatched grafts were likely to be rejected and/or cause severe GVHD. Use of HLA matched sibling donors reduced but did not remove this risk which was higher when non-sibling HLA matched family donors were used. Use of unrelated HLA matched donors (MUD) increased GVHD risk above that. Haploidentical donors, i.e., those with one of their two HLA haplotypes inherited from a parent, especially the mother, was started in the 1990s and has been substantially increased as methods for abrogating or treating GVHD have improved. Such a procedure has become more common, especially after the development of conditioning regimens involving cyclophosphamide (21) that appear effective at generating the early expansion of regulatory T cells.

Haploidentical transplantation has provided a unique platform for experimental tolerogenic strategies, with several studies providing convincing evidence that, at least when using the most appropriate donor, the outcome can be very good (22). A recent retrospective study (23) has convincingly documented that it is the patient and disease rather than donor features that affect survival of these patients. However, it is important to acknowledge the fact that the technique of haploidentical transplantation exposes patients to delayed immune reconstitution thus potentially limiting some of the benefits.

The use of bone marrow cells and G-CSF mobilized bone marrow cells as sources of HSC have been modified in a number of ways in attempts to reduce GVHD. Complete removal of T cells can be effective, but leukemic patients then have higher relapse rates, due to the removal of GVL effectors. Reduction in the number of contaminating T cells can help, but is difficult to titrate. Mitigating the risk of relapse, donor lymphocyte infusions (10) have been used and these, following HSC transplantation, have provided long-term curative treatment, particularly for chronic myeloid leukemia. Since T cells in these donor inocula are long lived and likely to contain a number of different clones with specificity for several transplantation antigens, mutant leukemic cells are likely to be targeted as they arise, a situation not replicated when targeted molecular therapy is given, directed against a determinant whose expression can be downregulated by mutation.

The use of cord blood as a source of HSC uncontaminated by primed T cells is practical only for child recipients, as single donations rarely contain sufficient stem cells to achieve engraftment. However, recent data suggests the opportunity to use aryl hydrocarbon antagonists to produce a robust expansion of hematopoietic stem and progenitor cells (24).

Autologous HSC avoided the risk of GVH and HVG reactions but its use in treatment of leukemias and other cancers was bedeviled by high relapse rates, as there can be no GVT effect. However, since the early 2000s the introduction of somatic gene therapy for inherited immunodeficiencies has been made possible by the identification of some of the relevant mutant genes, and methods for transducing corrected copies of them into autologous HSC *ex vivo* before transplanting them into the patient pre-treated to provide "space" for the newcomers. There are still issues that can limit the applicability of the gene therapy approach. On one side the modification of HSCs may reduce their capacity to engraft, whilst on the other the modification strategy may require the selection of the gene-corrected cells, thus impacting on the cell yield required to be efficiently transplanted (25).

Based on the notion that it is the graft-vs.-tumor effect that secures long-term eradication of the underlying malignancy, the conditioning regimens used to prepare patients for transplantation have been radically revisited. Whilst radiation was the main component of the pre-transplant conditioning because of its efficacy in eliminating replicating cells, other milder approaches have been used since the end of the 90s. Chemotherapeutic agents are now being used at doses by far lower than before, thus reducing toxicity and eventually reducing the frequency of GvHD that is largely affected, not only by the transplantation antigens but also and perhaps more importantly, by the cytokine storm induced by the tissue damage consequential to chemo/radiotherapy (26). Furthermore, it was shown that the use of cyclophosphamide soon after HSC infusion could mitigate the incidence of GvHD by increasing the number of regulatory T cells (27).

Unfortunately, GvHD remains the most dreadful complication of allografting and when refractory to steroid treatment the associated mortality is dismal. Cellular therapies may provide an alternative to traditional immunosuppressive approaches because they may provide an immunological reprogramming of the patient's inflammatory environment. Important milestones in this direction have been provided by the use of regulatory T cells (28) and mesenchymal stromal cells (MSC). Initially identified as tout-court immunosuppressants (29), MSC have been recently shown as effective at reprogramming the recipient phagocytic system to control unwanted inflammation (30). This has transplanted into very encouraging clinical experience (31).

HEMATOLOGICAL AND OTHER DISEASE CANDIDATES FOR HSC TRANSPLANTS

Leukemias, bone marrow failures, hemoglobinopathies (e.g., thalassemia, sickle cell disease) and immunodeficiencies have been mentioned, and advances with these could lead to HSC transplant-based treatments for solid tumors and other genetic diseases (e.g., lysosomal storage disease) as well as autoimmunity.

Reducing the incidence and severity of GVHD following HSC transplant remains the biggest challenge for both existing patients and the possibility of extending this treatment to additional diseases.

AUTHOR CONTRIBUTIONS

ES and FD drafted the review together. ES concentrating on the historical background and FD on the clinical aspects.

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frontiers in Immunology

REVIEW
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Immunology's Coming of Age

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This treatise describes the development of immunology as a scientific discipline with a focus on its foundation. Toward the end of the nineteenth century, the study of immunology was founded with the discoveries of phagocytosis by Elias Metchnikoff, as well as by Emil Behring's and Paul Ehrlich's discovery of neutralizing antibodies. These seminal studies were followed by the discoveries of bacteriolysis by complement and of opsonization by antibodies, which provided first evidence for cooperation between acquired and innate immunity. In the years that followed, light was shed on the pathogenic corollary of the immune response, describing different types of hypersensitivity. Subsequently, immunochemistry dominated the field, leading to the revelation of the chemical structure of antibodies in the 1960s. Immunobiology was preceded by transplantation biology, which laid the ground for the genetic basis of acquired immunity. With the identification of antibody producers as B lymphocytes and the discovery of T lymphocytes as regulators of acquired immunity, lymphocytes moved into the center of immunologic research. T cells were shown to be genetically restricted and to regulate different leukocyte populations, including B cells and professional phagocytes. The discovery of dendritic cells as major antigen-presenting cells and their surface expression of pattern recognition receptors revealed the mechanisms by which innate immunity instructs acquired immunity. Genetic analysis provided in-depth insights into the generation of antibody diversity by recombination, which in principle was shown to underlie diversity of the T cell receptor, as well. The invention of monoclonal antibodies not only provided ultimate proof for the unique antigen specificity of the antibody-producing plasma cell, it also paved the way for a new era of immunotherapy. Emil Behring demonstrated cure of infectious disease by serum therapy, illustrating how clinical studies can stimulate basic research. The recent discovery of checkpoint control for cancer therapy illustrates how clinical application benefits from insights into basic mechanisms. Last not least, perspectives on immunology progressed from a dichotomy between cellular-unspecific innate immunity and humoral-specific acquired immunity, toward the concept of complementary binarity.

Keywords: antibody, cytokine, dendritic cell, immunology, lymphocyte, macrophage, phagocytosis, recombination

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INTRODUCTION

In this treatise, I describe growth and maturation of immunology as a scientific discipline built on both basic research and medical application. Although I emphasize the birth of immunology and early decades of its evolution, I stress that immunology in its full maturity remains equally integrated in both basic and clinical research.

Immunology started in the last quarter of the nineteenth century with two major discoveries. The first of these was Elias Metchnikff's (1845–1916) identification of phagocytic cells, which engulf and destroy invading pathogens (1). This laid the basis for innate immunity. The second discovery was Emil Behring's (1854–1917) and Paul Ehrlich's (1854–1915) identification of antibodies, which neutralize microbial toxins (1, 2). This became the basis for acquired immunity. These findings also led to the distinction between cellular and humoral immunity. For obvious reasons, humoral immunity was often considered synonymous with acquired immunity, whereas cells were considered tightly linked to innate immunity. This was overlaid by a further segregation between the unique antigen specificity of the acquired arm vs. the non-specific innate arm of the immune response (**Figure 1**). This dichotomous view led to some confusion and controversy and it took some time until it transformed into a perspective of complementary binarity considering innate and acquired immunity as interactive partners. Today the two arms of antigen-specific acquired and antigen-nonspecific innate immunity are best viewed as a ying–yang concept, with highly intertwined, partly overlapping, and mutually beneficial activities. Further highly valuable information on the highlights of immunology in its nascence can be found in the many publications of A. Silverstein of which I only cite his major treatise (3).

From its birth, immunology was at the heart of biomedical research providing both crucial information on basic biological processes and on clinical application. This was recognized by the first ever Nobel Prize in Medicine awarded in 1901 to Emil Behring "for serum therapy in therapeutic medical science," (4) and also by the most recent Nobel Prize 2018 to honor the "discovery of cancer therapy by inhibition of negative immune regulation" by Jim Allison (1948–) and Tasuku Honjo (1942–) (5). Whilst Behring's discovery illustrates how medical application can stimulate basic research, the discoveries of Allison and Honjo epitomize clinical application as the result of in-depth understanding of basic biological mechanisms.

ACT I: THE FOUNDATION OF IMMUNOLOGY

Immunology emerged as an academic discipline in its own right out of the fertile soil of medical microbiology (6). The discoveries of Louis Pasteur (1822–1895), which confirmed and completed the germ theory of infectious diseases as well as Robert Koch's (1843–1910) meticulous studies on the etiology of infectious diseases, notably tuberculosis, raised a question of fundamental importance: Is the host a helpless prey of pathogenic microbes or is it equipped with an efficient defense mechanism to combat its invaders? Both Pasteur and Koch favored the notion that the host was defenseless. However it was Metchnikoff, at the Pasteur Institute in Paris since 1888, who earlier discovered the critical role of phagocytosis and intracellular killing in host defense (1), and it was Behring and Ehrlich, young independent researchers at Koch's institute for Infectious Diseases in Berlin, who identified antibodies as crucial counterparts to the toxic activities of bacteria (1, 2). We now know that the outcome of infection depends on close interactions between pathogen and host factors, probably best described by the term infection biology.

When Koch embarked on the next step in his career in Berlin in 1878, the pathologist Rudolf Virchow (1821–1902) was the most eminent professor at the Charité clinics (6). Virchow is the founder of cellular pathology, which assumes that all diseases are the result of malfunctioning of our body's cells (7). Hence, Koch's ideas on the etiology of infectious diseases seconded by the germ theory of Pasteur were highly criticized by Virchow. Ultimately, Koch's observations, well-supported by experimental evidence, became the accepted paradigm. According to the American physicist and philosopher, Thomas Kuhn (1922–1996), normal science progresses as long as available evidence can be accommodated in the existing paradigm (8). Once anomalies accumulate from scientific research that can no longer be integrated in an existing paradigm, the time is ripe for a paradigm shift (8). Koch and Pasteur introduced a paradigm shift by demonstrating that exogenous invaders can cause certain diseases, beyond those diseases caused by dysfunctional cells. Yet, they both largely overlooked the role of host immunity as important defense mechanism. This paradigm shift was initiated by Metchnikoff, Behring, and Ehrlich. Today we understand infectious diseases as the outcome of a crosstalk between host and pathogen. We also now know that immunology has more roles to play than only pathogen defense, such as surveillance of malignant cells. Moreover, a dysfunctional immune system results in allergy, autoimmunity or chronic inflammation thereby illustrating it as a double-edged sword.

Phagocytosis

Metchnikoff was born in 1845 in a part of Russia, which now belongs to the Ukraine (9). He studied zoology and soon became a traveling scientist. Notably, when working at the Zoological Station in Naples he studied simple organisms and identified specialized cells dedicated to nutrient uptake. These nutrients could be contained in particles and thus the concept of phagocytosis was conceived as a process of uptake of particles or microbes rich in food. Moreover, in his experiments with starfish larvae in Messina in 1883, Metchnikoff found that phagocytic cells were highly motile and migrated to sites of foreign insult (10). He later wrote about these groundbreaking observations:

> "… I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful starfish larvae as transparent as water. I was too excited to sleep that night in the expectation of the result of my experiment and very early the next morning I ascertained that it had fully succeeded. That experiment formed the basis of phagocyte theory to the development of which I devoted the next 25 years of my life …" (11).

Indeed, Metchnikoff changed his scientific interests from zoology to pathology and in this way became one of the first immunologists. He discovered phagocytes in vertebrates and began analyzing phagocyte functions in infectious diseases, such as anthrax, sepsis, and tuberculosis (**Figure 2**). Based on these

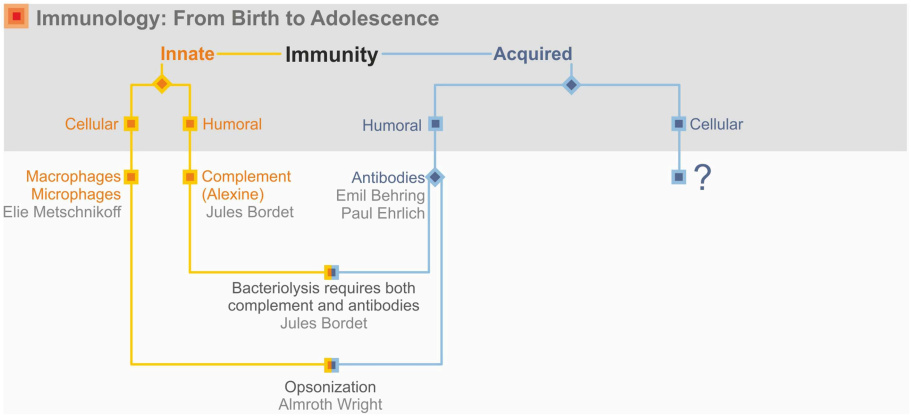

FIGURE 1 | Immunology's early days.

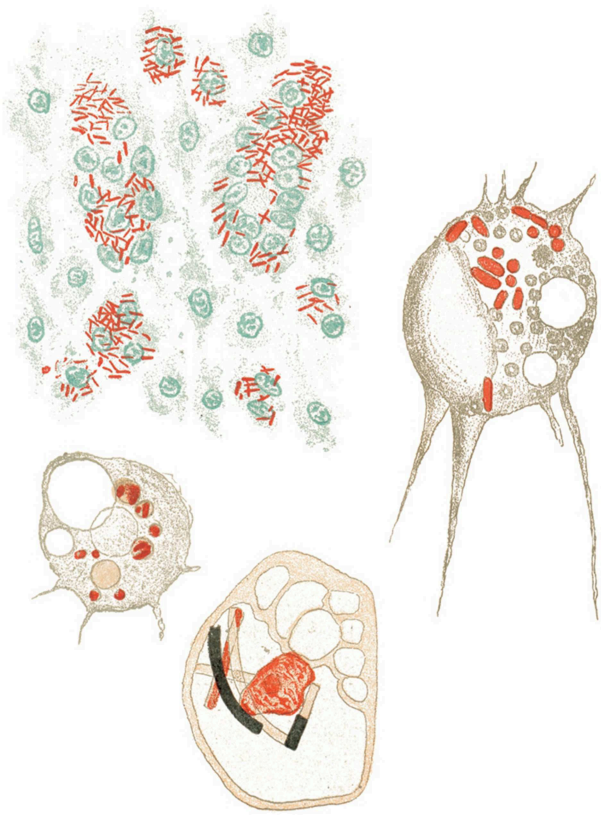

FIGURE 2 | Metchnikoff's view on phagocytosis of different bacterial pathogens (12).

studies, he distinguished macrophages from microphages (which we now call neutrophils) according to the form of their nucleus:

> "… I suggest calling all elements macrophages, which generally possess a simple non-polymorphic nucleus that is round or frequently oval. … as microphages I call smaller amoeboid cells, which can be easily stained, with a largely polynuclear and fragmented nucleus and faint protoplasm...." (13).

Serum Therapy and Antibodies

Behring was born in the German province of Prussia, now part of Poland, in 1854 (14). He studied medicine at an army academy and soon became interested in studies on the curative activity of disinfectants in bacterial infections. During his experiments on antiseptic activity of small molecules, together with the Japanese guest researcher Shibasaburo Kitasato (1853–1931) at the Institute for Infectious Diseases in Berlin, he discovered that serum from infected animals contained antibacterial activity that was specific for the infectious agent (15). Essentially, the activity was directed against the bacterial toxin. Whilst the joint paper of Behring and Kitasato mostly focused on tetanus and its toxin, the single-authored paper by Behring published shortly thereafter, described protection against diphtheria and its toxin by antisera (15, 16). Soon these animal experiments were translated into a human study, which revealed that serum therapy protected against diphtheria when given during early stages of infection or even during disease. Behring joined forces with industry to produce large doses of antisera for human use, thus embodying the translational immunologist with great interest in medical application (**Figure 3**). His serum therapy was a breakthrough and honored by the first ever awarded Nobel Prize in Medicine in 1901 (4).

Serum therapy was more than just a curative method. It also provided supportive evidence for the idea that the cause of infectious disease is highly specific and that this specificity is linked to toxins produced by the etiologic pathogen. As a corollary, the cure of the specific disease was accompanied by a specific poison-averting (antitoxic) agent, which circulates in blood and can offer specific protection against the toxin in other individuals (15–17).

Despite all the honors he received, Behring was not fully satisfied with passive vaccination. It took him some 20 years to solve the issue of active vaccination (18). In 1913, at the Congress for International Medicine in Wiesbaden, Germany,

FIGURE 3 | Large-scale production of serum against diphtheria toxin.

Behring gave a remarkable presentation, which the newspaper "Vossische Zeitung" (April 18, 1913) described quite aptly:

> "At today's discussions, Behring appeared as lively as ever and reported on a new protective agent comprising a mixture of diphtheria toxin and anti-toxin. This agent was harnessed for treating individuals at risk prophylactically. It was found that first the agent was completely innocuous, and second that the appearance of true protection could be demonstrated by the formation of sufficiently high abundance of protective agents in the blood of immunized individuals who all remained free of diphtheria" (14).

In order to neutralize the diphtheria toxin, Behring generated antigen-antibody complexes, which stimulated production of toxin-specific antibodies in the immunized host. This was an important, but still suboptimal start toward active vaccination against bacterial toxins. It was the French researcher, Gaston Ramon (1886–1963), who ultimately introduced detoxification by formaldehyde for low-cost production of safe vaccines against diphtheria and tetanus, and aluminum hydroxide as adjuvant for potent immunization (19, 20).

Whilst Behring was a translational immunologist, who contributed significantly to basic immunology, Ehrlich was most interested in the in-depth understanding of basic mechanisms underlying immunity, and contributed profoundly to the clinical development of serum therapy. Indeed it was Ehrlich whose contribution made large-scale production of antisera of reproducible quality possible. By working out "a new and more accurate method for determining the value of the serum and to study the complex relations which govern the neutralization of toxin and antitoxin," he could show that "… the immunity unit is no longer an arbitrary concept, but is an exactly determinable quantity and one therefore which can be reproduced afresh at any time …" (21). Ehrlich was therefore the first to provide the basis for a quality control measure of a biological. At those times, this was urgently needed because of widespread state-controlled compulsory vaccination against smallpox.

Yet, Ehrlich became most famous for basic research of, and stimulating ideas on, how the immune system works. In his MD thesis, Ehrlich described mast cells which, as we now know, are critical effectors of allergy (22). But his most important findings are related to antibodies. He foresaw that antigens, such as toxins, stimulate the production of specific antibodies. Interestingly, similar to Metchnikoff, Ehrlich assumed a nutritional point of view (22). Different cells need different kinds of nutrients and hence Ehrlich postulated specific receptors as being responsible for nutrient uptake. From this he concluded that the cell receptor specific for a given toxin should fulfill similar criteria. Because of the sheer abundance of toxins generated during infection, more specific receptors are produced and are ultimately secreted into the serum (**Figure 4**). In the Croonian Lecture given in 1900 at the Royal Society, Ehrlich reflected on his ideas as follows:

> "… the first stage in the toxic action must be regarded as being the union of the toxin …. to a special side chain of the cell protoplasm. … the side chain involved, so long as the union lasts cannot exercise its normal physiological nutritive function. …. such an excess of side chains is produced that to use a trivial expression, the side chains are present in too great quantity for the cell to carry and are, after a manner of secretion, handed over as superfluous ballast to the blood …" (23).

Essentially this is the core message of the side chain theory for which Ehrlich is most renowned. But Ehrlich was far more productive. He showed that the milk of breastfeeding mothers carries antibodies beneficial to the suckling infant, thus providing the child with a high degree of immunity (24). He speculated

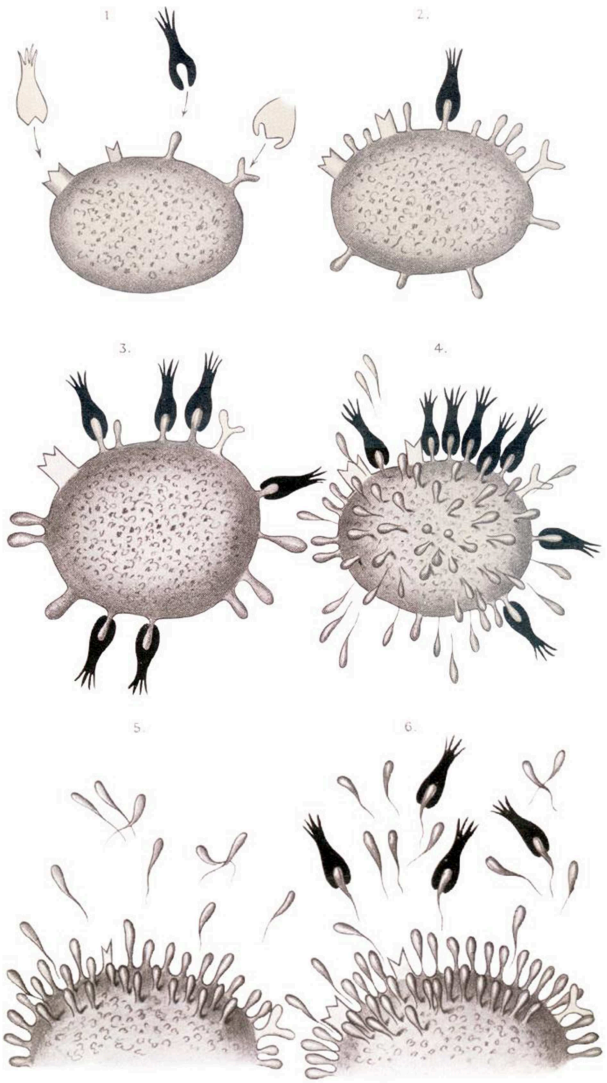

FIGURE 4 | Ehrlich's view on antibody secretion to different antigens (23).

on the role of tolerance to self and the risk of autoimmunity and coined the well-known term "horror autotoxicus" (24). He revealed several biological features of complement, which was originally discovered by the German scientist, Hans Buchner (1850–1902), and the Belgian researcher, Jules Bordet (1870–1961), who termed it alexine (25). Ultimately, however, the term complement created by Ehrlich prevailed. Bordet and Buchner had already shown that alexine was heat-labile (25–27). Buchner used serum from non-immunized animals, whereas Bordet included serum from immunized animals in his studies and so distinguished the heat-labile alexine from the heat-stable antibodies. Ehrlich, together with his colleague Richard Pfeiffer (1858–1945), further defined the activities of antibodies and complement by mixing untreated and heat-inactivated serum. In his own words, Ehrlich summarized this finding: "The two substances are (i) the specific immune body produced by immunization and (ii) a substance which usually is thermo-labile, contained even in normal serum" (28).

In 1908 Ehrlich and Metchnikoff were jointly awarded the Nobel Prize in Physiology or Medicine "in recognition of their work on immunity" (29). Bordet was honored "for his discoveries relating to immunity" with the Nobel Prize in 1919 (30).

The interaction of complement and antibodies was the first dent in the dichotomous view of immunity (**Figure 1**). Complement was part of the innate immune response and hence non-specific. But it was humoral. Thus, the exclusive association of innate immunity with cells had become obsolete. More importantly, specific antibodies cooperated with non-specific complement.

The dichotomous view of immunology was further softened by the experiments of the English scientist, Almroth Wright (1861–1947), who showed that antibodies can specifically facilitate phagocytosis of bacteria (31, 32). This is of particular importance for efficient defense against bacterial pathogens which evade phagocytosis, such as encapsulated bacteria (pneumococci, meningococci and gonococci). His finding revealed that for some diseases, specific antibodies are needed to interact with phagocytes for optimal host defense (31, 32). For the first time therefore, specific humoral factors of the acquired immune response (antibodies) were shown to collaborate with non-specific cognates of the cellular innate immune response (macrophage and neutrophils). This was another call for complementary dualism rather than dichotomy between innate and acquired immunity. The findings of Wright caught the interest of George Bernard Shaw (1856–1950), who described the potential of phagocytes for cellular therapy of disease. In Act I of "The Doctor's Dilemma," he writes: "There is at bottom only one genuinely scientific treatment for all diseases and that is to stimulate the phagocytes." During the play, however, the risk of adverse events of such therapy is increasingly recognized and culminates in the question: "Have we overstimulated the phagocytes? Have they not only eaten up the bacilli but attacked and destroyed the red corpuscles, as well?" Adoptive phagocyte therapy never made it into the clinics as an immunologic treatment regimen.

ACT II: IMMUNOCHEMISTRY AND CLINICAL IMMUNOLOGY

Immunochemistry

During the first half of the twentieth century, immunologists focused on clinical observations and even more on immunochemistry, which could build on a much broader armamentarium of technical tools. Immunochemistry found its culmination in the discovery of the chemical structure of antibodies (**Figure 4**). This was accomplished independently by the British chemist, Rodney Porter (1917–1985), and the US chemist, Gerald Edelman (1929–2014), in the late 1950s to early 1960s (33, 34). Their work was honored by the Nobel Prize in 1972 (35). The Austrian Karl Landsteiner (1868–1943), first working in Europe and since 1923 in the US, developed the carrier hapten concept by coupling small aromatic molecules to

proteins (36). He showed that the small residue—the hapten—is recognized by antibodies, and therefore serves as epitope, and that the protein serves as carrier to provide the immunogenicity needed for successful stimulation of an antibody response (37, 38). Since the studies of Jacques Miller (1931–), Henry Claman (1930–2016) and others, we know that the antibody response involves B lymphocytes for the recognition of the hapten and T lymphocytes for the recognition of the carrier.

Hypersensitivity Reactions

Landsteiner is probably best known for the discovery of the ABO major blood group system (39). Working at the time in Vienna, he found that mixing blood of two different individuals resulted in clumping of red blood cells. Based on this finding, he developed a technique for the serologic differentiation of erythrocytes, which allowed him to identify the different blood groups of the ABO system. This discovery was honored by the Nobel Prize in 1930 (40). Ten years later, and together with Alexander Wiener (1907–1976), Landsteiner discovered a second important blood group, called Rhesus (Rh), named after their original discovery with erythrocytes in Rhesus monkeys (41, 42).

Landsteiner's discovery of so-called isoagglutinins—the antibodies responsible for clumping of erythrocytes when mixed with serum from a donor of a different ABO blood group—were criticized by Paul Ehrlich who considered this finding contradictory to his proposed "horror autotoxicus." Yet, increasing evidence arose that horror autotoxicus, i.e., autoimmune attack against host cells or molecules was not an absolute no-go for the immune system. It became clear that antibodies do not only perform beneficial functions. That aberrant antibody responses could lead to hypersensitivity reactions was first shown by the French clinician Charles Richet (1850–1935) in 1902 (43), who was awarded the Nobel Prize for his research on anaphylaxis in 1913 (44). The term anaphylaxis was coined by Richet to describe harmful reactions, which were later shown by the Japanese immunologist Kimishigi Ishizaka (1925–2018) and his wife Teruko (1926–), to be mediated by antibodies of the IgE isotype (45). One year after Richet's discovery, the French researcher, Maurice Arthus (1862–1945), described a similar yet distinct type of reaction which he induced experimentally by local injection of antigen into the skin of an individual previously immunized with the same antigen (46). In contrast to the reaction described by Richet, this one was mediated by immune complexes and involved complement. With serum therapy against diphtheria and tetanus broadly applied, numerous individuals received serum from horses in which the antiserum had been generated. In 1905, the clinicians, Clemens von Pirquet (1874–1929) from Austria, and Béla Schick (1877–1967) from Hungary, together observed that multiple injections of such serum could result in serum sickness due to the formation of immune complexes (47). They termed this type of reaction "allergy," which has come to be applied in a broader sense. Yet, another hypersensitivity reaction was first observed by the Japanese physician, Hakaru Hashimoto (1881–1934), in 1912 (48): "Hashimoto's thyroiditis" turned out to be an autoimmune disease partially mediated by IgG antibodies, which facilitate damage by phagocytes and NK cells. This type of hypersensitivity is also the basis of erythrocyte damage after blood transfusion, e.g., from ABO-disparate donors. At Rockefeller University, Karl Landsteiner together with the American researcher, Merrill Chase (1905–2004), studied the tuberculin reaction first described by Robert Koch and demonstrated that this reaction can be adoptively transferred by cells of an immune animal but not by serum (49). As we know now, the "delayed-type hypersensitivity" reaction mostly involves T lymphocytes.

The four different types of hypersensitivity were categorized by the UK physicians, Philip Gell (1914–2001), and Robin Coombs (1921–2006), in 1963 (50). In this categorization, type I hypersensitivity is the typical IgE-mediated allergy first described by Richet; type II is IgG plus complement-mediated destruction of host cells; type III is mediated by immune complexes such as the Arthus reaction; and type IV is the delayed-type hypersensitivity reaction, including the tuberculin reaction and contact dermatitis. Hashimoto's thyroiditis, originally considered type II, is now known to be a mix of type II and type IV, i.e., it is antibody- and T cell-mediated.

ACT III: THE RISE OF IMMUNOBIOLOGY

Transplantation Biology

The 1950s to 1960s witnessed a marked shift in priorities from immunochemistry to immunobiology (**Figure 5**). In fact, studies on transplant rejection preceded and prepared the ground for immunobiology. The US geneticist George Snell (1903–1996), based on his studies with inbred mouse strains, elegantly demonstrated that distinct genes within the major histocompatibility complex (MHC) were responsible for transplant rejection (51). The French clinician, Jean Dausset (1916–2009), discovered the human MHC, also named human leukocyte antigen (HLA), on the basis of family studies (51). A somewhat more direct link to immunobiology was provided by the Venezuelan-born US scientist, Baruj Benacerraf (1920–2011), who identified the immune response genes within the MHC locus (51). In 1980, Snell, Dausset and Benacerraf were honored by the Nobel Prize "for their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions" (52). Later the Australian researcher, Peter Doherty (1940–), and the Swiss researcher, Rolf Zinkernagel (1944–), would broaden this perspective by showing that the MHC is crucial for antigen recognition by T lymphocytes, the cells that would become the dominant research target in the second half of the twentieth century.

Antibody Specificity Revisited

The Australian virologist, Frank Macarlane Burnet (1899–1985), and the UK biologist, Peter Brian Medawar (1915–1987), received the Nobel Prize in 1960 "for their discovery of acquired immunological tolerance" (53). It was they who provided first evidence that the horror autotoxicus, envisaged by Paul Ehrlich, was not prefixed but a matter of education. Medawar had shown that transplant rejection could be prevented by transferring cells from an unrelated donor during neonatal life (54, 55). Cells from the same donor were later accepted by such mice showing that during fetal and neonatal development

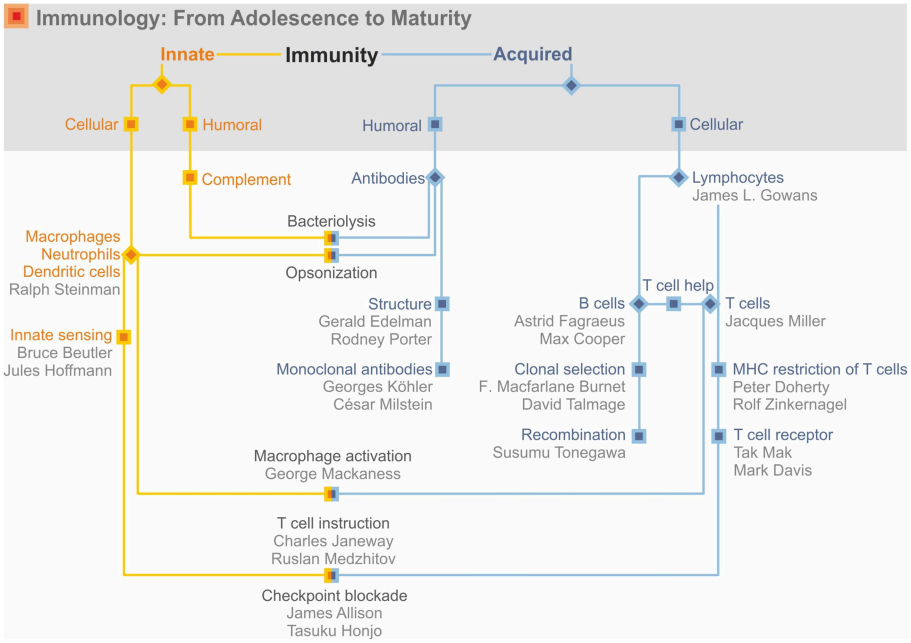

FIGURE 5 | Immunology: from adolescence to adulthood.

the immune system "learned" to accept self. Indeed it was Burnet who outlined the concept of "self vs. non-self" (55). Although his concept remained speculative and was questioned because of the occurrence of autoimmune diseases, it proved to be a valid theory of immunobiology even though—as with many biological issues—it was not absolute. In fact, impact of "self vs. non-self" on immune tolerance remains a matter of controversial discussions—not the least after the realization that self/non-self discrimination is not only a matter of the acquired but also of the innate immune response (see below). Burnet's interests were much broader. Originally a virologist who became an immunologist, he readily used tools of virology to interrogate the immune system. He is probably most famous for postulating the "clonal selection" theory, which again had been triggered by Paul Ehrlich (56). Although Ehrlich's side chain theory held that antibody specificities of all kinds were present before antigen encounter, according to Ehrlich numerous specificities could be expressed by a single cell depending on its requirement for specific nutrients (see **Figure 4**). This assertion, however, was questioned during the area of immunochemistry when a chemical explanation was sought for a biological question. Several researchers including the US Nobel laureate of 1954 and 1963, Linus Pauling (1901–1994), claimed that the structure of the antigen would determine the specificity of its corresponding antibody (57). In the "template hypothesis," the antigen binding site was the result of a specific chemical formation around a foreign entity. With the understanding that the three-dimensional structure of a protein is strongly determined by its amino acid sequence, this became a matter of impossibility.

The Danish immunologist, Niels Jerne (1911–1994), who received the Nobel Prize in 1984 (58), postulated a more biologically oriented hypothesis, namely that various antibody specificities existed prior to antigen encounter (59). This was then refined by Burnet and independently by the US immunologist David Talmage (1919–2014), who both proposed a selection process for the specific antibody-producing cell (56, 60). Thus, Ehrlich was right in assuming the preexistence of antibody specificities before a foreign antibody arrived, but he was wrong in assuming that one cell would express numerous specificities. Elegant studies by the Australian immunologist, Gustav Nossal (1931–), partly together with US Nobel laureate of 1958 Joshua Lederberg (1925–2008), provided strong evidence that a single cell produces an antibody of unique specificity (61, 62). Under the influence of the specific antigen, the antibody-producing cells expand numerically and produce more antibodies of the same specificity. Hence, interest in antibodies shifted from chemical structure to biological understanding of the generation of specificity, i.e., on the antibody-producing cell.

Lymphocytes as Masters of Ceremony

The major cell type of the acquired immune response, however, was still missing (**Figure 5**). It was the Australian immunologist, Jacques Miller (1931–), who discovered the role of the thymus in the development of a specific lymphocyte population; this finding led to the identification of T lymphocytes as major regulators of the acquired immune response (63). Independent from Miller, the US transplant immunologist Robert Good (1922–2003) characterized the role of the thymus and other lymphoid organs in the generation of different lymphocyte populations (64, 65). At about the same time, the UK immunologist, James Gowans

(1924–), had shown that the lymphocyte population was able to recirculate through the body and enter the different tissue sites—an important and necessary feature for T lymphocytes which mediate cellular immunity and hence depend on cell–cell contact (66). The producers of antibodies had been identified earlier, namely in 1940 by the Swedish researcher, Astrid Fragaeus (1913–1997), as plasma cells (67, 68). Her work as well as that of the US immunologist, Max Cooper (1933–) then led to the revelation that plasma cells are derived from B lymphocytes which develop in the Bursa fabricii in birds and in the bone marrow in mammals (64, 65, 69).

Now the major cells of the acquired immune response had been identified and immunologists increasingly focused on their biological functions (**Figure 5**). Henry Claman (1930–2016) was probably the first to provide compelling evidence that T lymphocytes and B lymphocytes collaborate in the generation of antigen-specific antibodies (70). Av Mitchison (1928–) showed that antibodies were specific for the epitope (Landsteiner's small residues—the haptens) and T cells for the protein carrier (71). The establishment of T lymphocytes and B lymphocytes as responsible cells of acquired cellular and humoral immunity, respectively, and their collaboration in shaping an optimal immune response laid the basis for the golden age of cellular immunity.

Following the footsteps of the founders of immunology, the Australian borne researcher working in the US, George Mackaness (1922–2007), extensively studied immunity against intracellular bacteria. He discovered the cooperation between specific T lymphocytes and mononuclear phagocytes. In this setting, antigen specific T cells stimulate increased antibacterial activities in macrophages which thereby change from a habitat for the intracellular pathogens to the major effectors of cell-mediated immunity against the infection (72).

Transplantation biology and immunobiology converged when Peter Doherty and Rolf Zinkernagel demonstrated that MHC molecules were not only responsible for transplant rejection, but for T-cell recognition of any type of antigen. Antigen recognition by T lymphocytes, therefore, was MHC-restricted and transplant rejection was just one special case (73). Their breakthrough work, honored by the Nobel Prize in 1996, was based on antigen recognition by cytolytic T lymphocytes, which kill virus-infected cells (73, 74). Soon these cells were characterized phenotypically as CD8 T cells, which were MHC I-restricted. CD8 T cell counterparts, the CD4 T cells, were MHC II-restricted and shown to activate other cells of the immune system, notably B cells and macrophages by means of soluble factors, the cytokines. Activation of macrophages increases antibacterial activities, which in turn allows macrophages to control intracellular bacteria, such as the causative agent of tuberculosis. B cell activation leads to the production of antibodies of different isotypes. CD4 T cells were also found to help CD8 T cells become killer T cells. The first molecularly defined T cell cytokine was interleukin-2 (IL-2), which was originally described by the US immunologist, Kendall Smith (1933–) (75). His findings paved the way for the discovery of numerous humoral mediators of T cell immunity. With the identification of many other cytokines, the concept of T helper 1 (TH1) vs. T helper 2 (TH2) cells was developed by the Canadian immunologist Tim Mosmann (1949–) and the US immunologist Bob Coffman (1949–) (76). CD4 T cells of TH1 type contribute to the cellular immune response by activating killer T cells and macrophages. IL-2 was identified as the major mediator of killer T cell activation and interferon-γ (IFN-γ), which had already been described earlier as immune IFN was shown to be critical for macrophage activation. In contrast, TH2 cells produce IL-4 and other cytokines, which stimulate B lymphocytes to mature to antibody-producing plasma cells. Early on it was recognized that the immune response is highly regulated and notably that a well-functioning immune response need not only be activated to combat an intruder, but also needs to be downregulated once the intruder had been eliminated. This led to the concept of a highly regulated immune response involving specific T cells with suppressive functions to avoid collateral damage. Early attempts to explain this issue postulated suppressor T cells which, however, did not stand the test of time. The more refined concept of the better defined subsets of regulatory T cells, however, provided compelling evidence for specific T lymphocytes which not only control immune responses after elimination of invading pathogens, but also prevent autoimmunity and maintain homeostasis (77).

Although the biological functions of T lymphocytes were increasingly better understood, their antigen receptors remained elusive until the 1980s. By using monoclonal antibodies, US immunologists, Pippa Marrack (1945–) and John Kappler (1943–) (in the mouse system) (78), and Ellis Reinherz (1950–) and Stuart Schlossman (1935–) (in the human system) (79), were able to phenotypically identify antigen-specific receptors on T lymphocytes. This was the first hint for the existence of the antigen-specific T cell receptor (TCR). Soon thereafter, genes encoding TCR chains were cloned by Tak Mak (1946–) in Canada and Mark Davis (1952–) in the US (80, 81).

The T lymphocyte system can thus also be viewed as a binary system (**Figure 6**). Lymphocytes segregate into B and T cells; T cells segregate into MHC I- and MHC II-restricted T cells of CD4 or CD8 phenotype, respectively; CD4 T cells separate into TH1 and TH2 cells; the vast majority of T cells express a T cell receptor composed of an α and a β chain, but a second T cell population exists, which expresses a T cell receptor comprising a γ and a δ chain. Again, support was withdrawn for a dichotomous view, in favor of a complementary dualism (**Figure 5**).

Recombination Generates Diversity

These important findings were preceded by the breakthrough discovery of the Japanese researcher, Susumu Tonogawa (1939–), then in Basel, Switzerland, who elucidated the mechanisms underlying the huge diversity of antibody specificities (82, 83). By then it was generally accepted: a single specific B cell was responsible for antibody production; diversity was generated prior to the first contact with antigen; a single B cell expresses a receptor with a unique specificity; contact with the homologous antigen stimulates selective expansion and differentiation of the specific B cell. Yet, one critical issue remained unsolved, namely that the number of possible antibody specificities exceeded the number of genes present in our

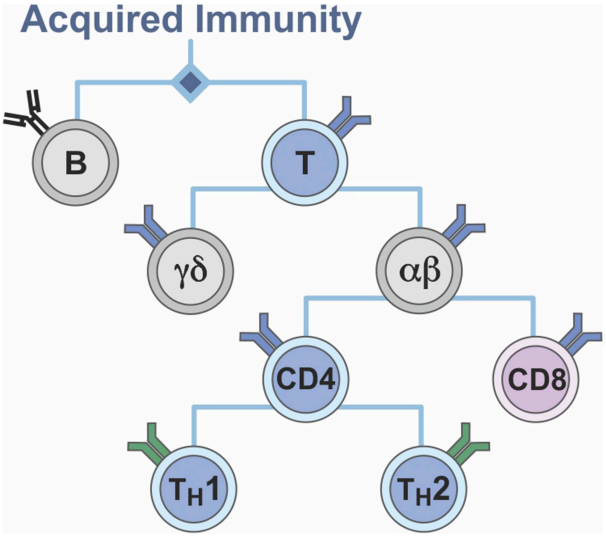

FIGURE 6 | Binarity of the T cell system.

body. The solution to this was identified by Tonegawa as the rearrangement of gene fragments. This recombination allows the generation of more than one million specificities which further increases numerically by additional mechanisms to up to some 10^9 specificities. Tonegawa was honored with the Nobel Prize in 1987 "for the discovery of the genetic principle for generation of antibody diversity" (84). Principally, antigen diversity of the T cell receptor is based on similar genetic mechanisms.

T-Cell Instruction by Antigen-Presenting Cells

In any case, the specificity of the acquired immune response and the multiple roles played by T cells more or less dominated immunobiology in the 1960s to 1990s. An influential researcher in the field of T cell immunology was Charles Janeway (1943–2003) from the US (85), who in a remarkable paper published in 1989 in the Proceedings of the Cold Spring Harbor Symposium, pointed to the widely underestimated role of the innate immune system (86). Prevailing opinion was that innate immune cells, notably macrophages and neutrophils, play an important effector role in host defense, under the guidance of T lymphocytes and their soluble products. Even though it was clear that T cells recognize antigens in the context of MHC presented on the surface of so-called antigen-presenting cells, these cells were viewed more as passive guides than active players. Janeway postulated the presence of pattern recognition receptors on antigen-presenting cells, which sense specific motifs of chemical products of bacteria and viruses and then instruct T cells about the different functions they should perform. Most compelling evidence for such an idea came from studies on the toll-like receptors (TLR) in mammals by the US geneticist Bruce Beutler (1957–), and in insects by the biochemist Jules Hoffmann (1941–) in France (87, 88). This led to the concept that different types of pathogens are sensed by pattern recognition receptors with specificity for microbe-associated molecular patterns. Beutler and Hoffmann jointly received the Nobel Prize in 2011 "for their discoveries concerning the activation of innate immunity" (89). The concept of sensing of microbial motifs (so-called pathogen-associated molecular patterns, PAMP) by innate receptors was soon broadened when similar mechanisms were found to be induced by host motifs (so-called danger associated molecular patterns, DAMP) which arise from insult to the host (90). In how far PAMP and DAMP influence immune tolerance by inducing danger associated non-self or self-signals to the induction of an acquired immune response remains a matter of controversial discussion (91, 92).

As early as the 1970s, the Canadian immunologist Ralph Steinman (1943–2011) at Rockefeller University, US, was engaged with defining the critical player in this concept: the dendritic cell (93). He demonstrated that dendritic cells are much more potent antigen presenters than macrophages, and that they are the major instructors of T cells regarding the type of pathogen they will encounter. Steinman was the third to be honored by the Nobel Prize 2011 "for his discovery of the dendritic cell and its role in adaptive immunity" (89). Sadly he could not accept the award in person because he passed away shortly before the ceremony. In conclusion, innate immunity plays a crucial role, from the beginning to the end of an immune response. In the beginning it acts via antigen-presenting cells, which not only stimulate antigen-specific T cells but also serve as instructors for the biological functions T cells have to perform. Toward the end, innate immunity takes care of effector functions, e.g., via professional phagocytes which eliminate invading pathogens.

Instruction of T cell functions strongly depends on cytokines, i.e., humoral factors. Thus, IL-12 induces TH1 cells whereas IL-4 directs TH2 cells. In fact, the first chemically defined cytokine was described by the US immunologist, Charles Dinarello (1943–) as a macrophage-derived product, which accordingly was later named IL-1 (94). IL-1 plays a role in the instruction of TH1 cells and serves as mediator of inflammation.

From Serum Therapy to Checkpoint Control

B cells stood in the shadow of T lymphocytes during the 1970s. The discovery by the Argentinian researcher, Cesar Milstein (1927–2002), and the German researcher, Georges Köhler (1946–1995), both working in the UK, brought them back to center stage. In 1984, both shared the Nobel Prize "for the production of monoclonal antibodies" (58). Obviously, this discovery had major implications. First, it allowed the ultimate proof for the production of an antibody with single specificity by a single plasma cell and second, it paved the way for a new era of immunotherapy. As a short reminder, the concept of acquired immunity started with antibodies and was intrinsically intertwined with the concept of serum therapy, for which Behring received the Nobel Prize in 1901. Now the tools for more precise passive immunization had been put on the table. This led to the development of a number of monoclonal antibody-based therapies for infectious diseases; currently, the focus of monoclonal antibody therapy is on immunomodulation. Thus,

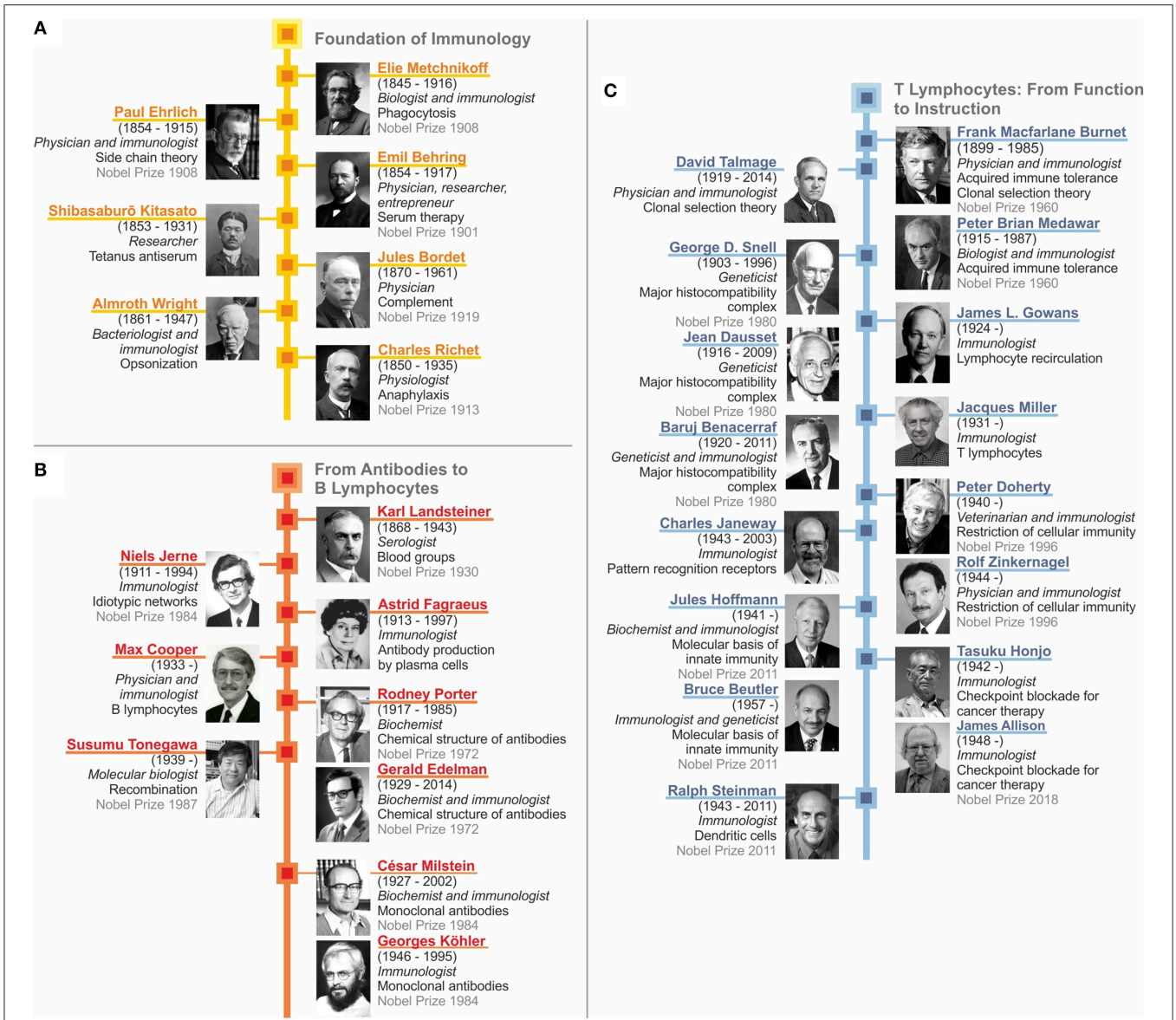

FIGURE 7 | Recap of immunology: from serum therapy to checkpoint control. **(A)** Foundation of immunology. **(B)** From antibodies to B lymphocytes. **(C)** T lymphocytes: from function to instruction.

cytokine-blocking monoclonal antibodies have been introduced in the treatment of chronic inflammatory diseases. Most notable are Infliximab and Adalimumab, which block the critical cytokine tumor necrosis factor-α (TNF-α) in Crohn's disease and rheumatoid arthritis, respectively (95, 96). A second important target of therapeutic monoclonal antibodies are surface-expressed molecules such as CD20 on B lymphocytes, which can be harnessed for treatment of non-Hodgkin's lymphoma such as Rituximab (97).

A major recent breakthrough has been the discovery of monoclonal antibodies which block checkpoint control. What does this mean? Regulation of T cell activity is not only a matter of cytokines but also of costimulatory molecules, which in addition to TCR recognition of antigen plus MHC as first signal, provide a second signal for T cells in stimulating their effector functions. Eventually, the immune response needs to be dampened. Once it has completed its task, e.g., after the elimination of an infectious agent, it needs to be tuned down to avoid or at least minimize collateral damage. Surface-expressed inhibitory molecules include CTLA-4 and PD-1 on T cells and their counterparts B7 and PD-L1 on antigen-presenting cells (98, 99). These counterparts are also expressed on many tumor cells, which block attack by killer T cells. Blockade of checkpoint control improves T cell responses and thereby allows elimination of certain tumor cells. This finding led to next-generation immunotherapies for certain cancers including

metastatic melanomas and non-small cell lung carcinomas. The highly promising checkpoint blockade for cancer therapy was honored by the Nobel Prize 2018 to the US immunologist Jim Allison (1948–) and Japanese immunologist Tasuku Honjo (1942–) "for their discovery of cancer therapy by inhibition of negative immune regulation (5)."

SHORT RECAP AND OUTLOOK

As we have seen, immunology as a scientific discipline was kick-started by two seminal discoveries: First, the role of phagocytosis performed by cells and second, the neutralization of bacterial toxins by antibodies. This led to the concept of dichotomous roles of antigen-unspecific innate immunity mediated by cells and antigen-specific acquired immunity mediated by humoral factors. This dichotomous concept converged with the identification of complement and opsonization, which linked innate and acquired immunity. Major early contributors are depicted in **Figure 7A**. The intermediate stage includes the discovery of different forms of clinical hypersensitivity emphasizing that the immune system also embodies detrimental functions. In parallel, immunochemistry reached its climax with the elucidation of the crystal structure of antibodies. Then immunobiology took over with the identification of lymphocytes and their segregation into antibody-producing B cells and plasma cells, as well as T cells, which function as central regulators of immunity (**Figures 7B,C**). TH cells were shown to control B lymphocytes, professional phagocytes and cytolytic T cells. Finally, this dysbalanced perception of acquired immunity dominating innate immunity was rectified by our increasing understanding of how antigen-presenting cells instruct the acquired immune response (**Figure 7C**). Today sufficient knowledge has been accumulated in immunology to devise sophisticated therapeutic approaches, such as checkpoint control for cancer treatment. Yet, in both basic and applied immunology, sufficient challenges persist which guarantee that our discipline will remain as vital as ever.

Importantly, the immune apparatus is increasingly seen as a highly diffuse organ comprising not only bone marrow, thymus and spleen, but also lymph nodes and lymphoid follicles which are spread throughout the body and interconnected by circulating leukocytes and soluble mediators. Accordingly, immune cells are imprinted by their organ of residence to adjust to the special regional needs. Reciprocally, immune cells impact on the tissue of their main residence. Moreover, our microbiome is increasingly viewed as a human organ vital to health and disease and tightly intertwined with the immune system. As a corollary, dysfunctions of regional immune responses underlie many organ-specific diseases. Future immunology will have to take into account an integrated view on these crosstalks at all levels from organs to tissues to cells to molecules. The enormous advances in high-throughput multi-omics technologies and bioinformatics allow studies on multiple levels of the immune response thus providing a wealth of data which will ultimately result in the construction of molecular multi-networks of the immune response under physiologic and pathologic conditions. Ultimately, this system biology approach will provide a far more comprehensive perspective of immunology which will generate new concepts for prevention and treatment of diseases that are refractory to current intervention strategies due to dysfunctional, insufficient or subverted immunity. Paul Ehrlich's dream of "magic bullets" will take a step closer to reality by the immunology of the future.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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A short perspective on the history of immunology is not a comprehensive account of all the different contributions to immunology. And by definition, a perspective is inherently biased. Hence, the views expressed here reflect my personal opinions and should not be taken as consensual reporting on the history of immunology. Wherever a Nobel Prize was awarded in recognition of an important breakthrough in immunology, I focused on the Nobel laureates' work. I am well-aware that with this approach, I may have missed important contributions by others. I apologize to all whose work I have omitted in my attempt to concisely summarize the history of immunology in this short overview. German citations have been translated freely into English by myself. I thank Alan Sher for many helpful comments on the manuscript, Marylu Grossman for excellent editorial support, Souraya Sibaei for excellent secretarial assistance and Diane Schad for superb graphics.

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frontiers in Immunology

GENERAL COMMENTARY
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Commentary: Immunology's Coming of Age

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A Commentary on

Immunology's Coming of Age
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The recent review by Stefan Kaufmann on "Immunology's Coming of Age" is an elegant historical outline of the evolution of Immunology with focusing on a particular perspective of the history of Immunology, that is Nobel Laureate contributions to the discipline. Immunology is a difficult discipline to survey. Even the best attempts would ultimately focus on some selected aspects. As such, it invites comments aiming to complement the presented history in the context of Immunology coming of age. It is the aim of our Commentary to add important research in the field of immunology to demonstrate that it has become a self-containing discipline.

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INTRODUCTION

Immunology is a rich discipline with successes and failures, with various scholarly works describing its origins and history that lend to our current understanding of immunological principles (1, 2). Still another perspective has been presented recently by Stefan Kaufmann emphasizing notable contributions acknowledged by the awarding of a Nobel Prize to outstanding investigators (3). While touching on extremely important developments, important contextual elements need to be mentioned to complement the presented history as important contributions are not always recognized by a Nobel Prize.

DISCUSSION

Antibody Recognition and Diversity

Saying that Immunology is an interdisciplinary science may no longer be entirely true since now it has also its own methods. The most prominent immunological paradigm is the concept of antibody. The specificity of antibodies is still an important question in immunology. Historically, the generation of diversity of antibodies was a hot discussed topic in the middle of the twentieth century initiated by the template hypothesis of Breinl and Haurowitz in 1930 (4), 10 years prior to Pauling's claim, cited in Kaufmann's review, that antibodies were made by folding newly synthesized nascent antibody polypeptide chains around the antigens, which serve as a template. Breinl and Haurowitz "thought that antibodies acquired their specificity for antigen by folding of the newly synthesized nascent polypeptide chain around the antigen" (5). The biochemical properties of antigen-antibody binding interactions were examined in more detail in the late 1930s by John Marrack (6). The biomolecule responsible for these actions was termed antitoxin, precipitin, and agglutinin. It was not known that all three substances were one entity. This was later demonstrated by Elvin A. Kabat showing the heterogeneity of antibodies through ultracentrifugation studies of

horses' sera. Similarly, an equally important milestone in the understanding of Immunological recognition was the x-ray resolution of a Fab antibody fragment (7) not recognized in the review and the founding of the definition of antibody diversity and its biological significance by Kabat (8, 9). This work provided a transforming view of antibody diversity and the molecular basis for antigen recognition (10).

Idiotype Hypothesis

Niels Jerne made several important contributions to Immunology. Niels Jerne's antibody selection theory is cited, but his more important contribution in the field of Immunobiology, the Idiotypic Network hypotheses, is not mentioned being essential for a historical record (11). He suggested that antibodies could be recognized as foreign, inducing other antibodies and thereby forming a network. Neglecting idiotype may be seen as more of a cultural aspect since it has not been accepted as a mainstream theory. Nevertheless, it has left a considerable imprint in immunological thinking. Recent reviews in Frontiers address the importance of the Idiotype concept in Immunology (12, 13). It might be argued that the Idiotypic Network hypothesis is the forerunner of present day ideas on the role antibodies plays in integrative Systems Immunology (14).

Selection

Positive and negative selection (of both T and B cells) as well as the practical and theoretical aspects of intravenous immunoglobulins are important Immunology discoveries. The term "tolerance" was first coined by Ray Owen in reference to a physiological state he observed in dizygotic twin cattle (15) as noted in a review of the historical record of immunological tolerance (16). Just like antibodies, the elucidation of the T cell structure was monumental (17, 18). This facet provided the backdrop of monumental studies by Ellis Reinherz, Phillippa Marrack, John Kappler, and James Allison. Checkpoint inhibitors, which are driving Immmunotherapy, owe their existence to the understanding of how T cells in particular function.

Natural Antibodies

Of no less importance is the regulatory and therapeutic potential of natural antibodies (19). Natural antibodies play an important role in the first line of defense and house keeping (20, 21). For a long period, natural antibodies were merely regarded as insignificant background of immunity. However, an early study in 1925 indicated that natural antibody in normal serum could neutralize bacteria (22).

Therapeutic Antibodies

With the discovery of immortalizing antibodies by Kohler and Milstein (23) opened a new drug class to treat infections, auto-immunities and other diseases (24, 25). In parallel intravenous immunoglobulin (IVIg) emerged as standard therapy of immunoglobulin deviancies, auto-immune reactions and in homeostasis (26–28). These translationary aspects of Immunology deserve to be noticed.

CONCLUSION

The History of Immunology began with Edward Jenner's discovery that vaccination protects against smallpox. Many scientists and discoveries have since lent to our understanding of how the immune system fights disease and sometimes causes disease as well to new classes of drugs. As we move closer to individualized medicine scenarios there will be a continuing need to understand and maybe redefine what came before and what will evolve in the discipline Immunology.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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MINI REVIEW
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Vaccine Evolution and Its Application to Fight Modern Threats

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Before the development of the first vaccine, infectious diseases were a major cause of death around the globe with life expectancy estimated to be <50 years. Three measures have helped to drastically reduce the burden of infectious diseases but only vaccines have proven to be able to eradicate infectious agents. Herein, we describe new methodologies that have paved the way for what is currently known as modern vaccinology and the use of vaccines to tackle antimicrobial resistance, the biggest global threat of our time.

Keywords: vaccines, infectious diseases, antimicrobial resistance (AMR), vaccine development, vaccinology

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THE BURDEN OF INFECTIOUS DISEASES BEFORE ANTIBIOTICS AND VACCINE INTERVENTION

Infectious diseases have always had a devastating impact on humankind. Some of the most catastrophic pandemics of our history include the Justinian plague (542-546 AD), which had a tragic toll of 100 million deaths, the bubonic plague (1347-50 AD), also known as the "Black Death," which erased one-third of the entire human population (1, 2), and more recently the "Spanish" influenza in 1918 which caused ∼50–100 million deaths worldwide reducing the European population by half (3–5). Before the introduction of effective preventive and therapeutic strategies, life expectancy was estimated to be <50 years and bacterial infections were the imperative toll setting this limit (6). This scenario changed with the introduction of three measures that helped to dramatically reduce the death burden caused by infectious diseases. The measures include hygiene, antibiotics, and vaccination (7, 8). The introduction of penicillin in 1929 (9), and its first use in humans a decade later (10), led to a dramatic reduction of mortality caused by infectious diseases. Unfortunately, in 1940 the first case of a penicillin resistant *E. coli* strain was documented and by the late 1960s over 80% of *S. aureus* strains acquired the same resistance (10–12). Therefore, despite the use of antibiotics resulted to be an outstanding first line of defense to treat infections, pathogens have shown to quickly acquire resistance phenotypes after only few years from their introduction (13). Vaccines, on the other hand, have only rarely shown to induce resistant phenotypes as they usually aim to elicit a multi-targets immune response and their prophylactic use reduces the likelihood of spreading resistant-conferring mutations (14). Indeed the smallpox vaccine introduced in 1796, and subsequently manufactured from infected calf skin (15), has led to the eradication of this infectious agent in 1988 (16, 17). Therefore, despite the fact that antibiotics and vaccines are pivotal interventions against infectious diseases, vaccination has been the sole intervention capable of eradicating an infectious agent and, given its potential, it can also be considered as the most appropriate solution against future global threats represented by infectious diseases (18–20).

REVERSE VACCINOLOGY AND THE DEVELOPMENT OF MODERN VACCINES

Since Edward Jenner first vaccinated an 8 year old boy in 1796 by inoculating fresh cowpox lesion matter (21), enormous leaps forward have been made in the field of vaccine development. Empirical approaches like attenuation and inactivation of microorganisms were the first steps forward to modern vaccinology (22). Recently, new technologies such as glycoconjugates and the introduction of novel vaccine adjuvants changed the field of vaccines, however the biggest change came with the first sequencing of the *Heamophilus influenzae* whole genome in 1995, a discovery that allowed the birth of "Reverse Vaccinology," a genome-based approach to vaccine development (23, 24). This approach, following the sequencing and analysis of the *Neisseria meningitidis* serogroup B strain whole genome, allowed the identification of novel candidates and the development of a four-component meningococcus B vaccine (4CMenB) (25, 26). This recently licensed vaccine has already shown incredible effectiveness in the UK with 82.9% protection against all MenB strains in infants (27). The evolution of vaccine development further moved forward with the advancement of new methodologies and technological breakthroughs. Indeed, in 2016 the "reverse vaccinology 2.0" entered the stage. With this approach, the human immune system is analyzed at a single cell level allowing the characterization of the antibody response like never before (28). The gain of knowledge acquired by this approach allows to rapidly identify highly immunogenic antigens to develop novel and more efficacious vaccine candidates. The RSV fusion protein (F) case is a major example of the phenomenal power of the reverse vaccinology 2.0. Indeed, human B cells were directly isolated from RSV convalescent donors and cultured to naturally produce human monoclonal antibodies (humAbs). Among all the antibodies screened for RSV neutralization *in vitro*, the humAbs named D25 resulted in the most potent antibody with a median half-maximal inhibitory concentration (IC50) of 2.1 ng/ml (100–150 times more than palivizumab, the only monoclonal antibody approved by the FDA for RSV prevention in infants) (29). Interestingly, D25 was not capable of binding to the RSV F-protein in its post-fusion conformation, the only vaccine candidate available at the time against RSV (30). Then, McLellan and coworkers had the brilliant intuition to test D25 complex with the RSV F-protein to perform structural studies. This experiment was paramount in solving the crystal structure of RSV F-protein in its pre-fusion conformation (preF) which in turn led to the design of a stabilized RSV preF molecule (30, 31). Following the production of a soluble preF reagent, numerous human neutralizing antibodies have been identified allowing a deep characterization of the antigen surface and the identification of two preF-specific antigenic sites that have shown incredible high neutralization potency (32). The effectiveness of the preF antigen has already been proven in different animal models (mice, rhesus macaques, and calves) further supporting the potential of RSV preF as an ideal vaccine candidate against this pneumovirus (10, 13). The power of reverse vaccinology 2.0 has allowed, in <5 years since preF stabilization, to start clinical trials that are currently on-going to develop the first vaccine against RSV (7). This approach, which has found broad applicability to fight viral infections, could also be considered as a key stratagem to tackle bacterial infections.

USE OF PEPTIDE-ANTIGEN DERIVED FOR GERMLINE TARGETING VACCINOLOGY

The production of germline-targeting (GT) antigens for vaccine development is another pivotal example that underlies the outstanding potential of reverse vaccinology 2.0. Indeed, the combined knowledge acquired by the identification and characterization of novel antigens plus the functional/genetic analysis of human monoclonal antibodies naturally produced by infected or vaccinated human donors, can be used to design antigen-derived peptides, capable of tailoring the antibody immune response. In case of highly variable pathogens such as HIV, the use of the whole antigen can result in a strain specific response, while the development of GT-antigens can lead to the elicitation of broadly neutralizing antibodies (bnAbs) capable of clearing multiple infective strains. This is a two-step approach which, using different rationally designed immunogens, aims to: (1) prime the germline precursor B cell of antibodies previously shown to possess broadly neutralizing activity; (2) shepherding the bnAb population by driving their maturation affinity toward the highly immunogenic epitope of interest. GT-vaccinology has

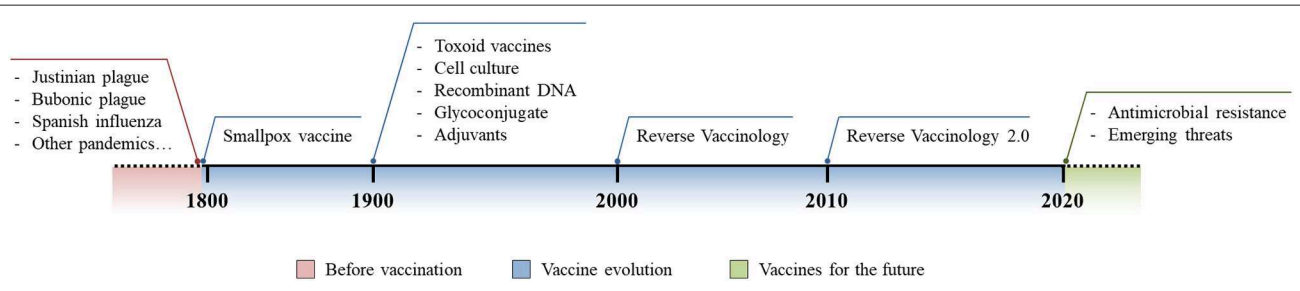

FIGURE 1 | Vaccine evolution. Schematic representation of the burden of infectious diseases before vaccination was introduced (red), technological and methodological advances in vaccinology following the introduction of the first vaccine (blue), and the future use and implementation of vaccine development to fight modern threats (green).

been used to elicit a specific class of HIV-1 gp120 CD4-binding site specific-bnAbs known as VRC01, through the use of engineered outer domain germline-targeting (eOD-GT) peptides (33). The interest to prime VRC01-bnAbs arises from their ability to mimic the CD4-binding to the gp120 receptor binding site and their capability to potently neutralize (median IC50 40 ng/mL) up to 98% of a large panel of global HIV-1 isolates (34, 35). An in-depth analysis of the VRC01 genetic features has shown peculiarities in this class of bnAbs. They classically derive from an extensively mutated (32–48%) VH1-2*02 heavy chain germline which pairs with light chains, presenting a rare five amino acid long CDR3 motif (usually QQYEF) (36). These analyses were paramount for the development of novel and potentially therapeutic candidates to fight HIV infections. Examples of the use of VRC01-bnAbs as a therapeutic tool are the monoclonal antibodies named VRC-HIVMAB060-00-AB (VRC01) and a FC-modified version of this latter named VRC01LS. These two bnAbs are currently under clinical investigation (NCT02568215, NCT02716675, and NCT02599896) evaluating safety and efficacy in reducing acquisition of HIV-1 infection (37–40). In addition to monoclonal antibody development and application, the knowledge acquired from these studies and the ability to selectively expand this class of bnAbs upon immunization (41), have allowed the development of specific peptides as vaccine candidates capable of shepherding the immune system toward a VRC01-like antibody response. The most promising candidate is the tailored immunogen named eOD-GT8 60-subunit self-assembling nanoparticle (eOD-GT8 60mer) (36, 42) which has shown superior affinity and breadth of binding to germline-reverted VRC01-like bnAbs (41).

The HIV case described above further confirms the outstanding power of reverse vaccinology 2.0. Indeed, in only 3 years since its design and stabilization (43), the eOD-GT8 60mer antigen is under investigation in a phase I clinical trial in healthy adults aimed at assessing safety, tolerability and immunogenicity of this germline-targeting immunogen (NCT03547245).

VACCINES FOR THE FUTURE: THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE

Despite antibiotics being the only lifesaving tool in fighting acute bacterial infection, as Stanley Falkow said (3), they are creating some problems of their own. In fact, the improper and excessive use of antibiotics has pressured bacteria to acquire antibiotic resistant phenotypes and this problem is currently growing out of control. Bacteria have shown several mechanisms to acquire antibiotic resistance and examples include the expression of β-lactamases, efflux pumps, modification of the cellular surface, and gene mutations to alter those molecules that are targeted by antibiotics (4). This phenomenon, known as antimicrobial resistance (AMR), is arguably one of the biggest threats that our world is facing today. Indeed, up to 700,000 deaths each year are AMR-related and these have been estimated to increase up to 10 million by 2050, exceeding the 8.2 million deaths per year caused by cancer today (8, 44). A solution to this alarming threat would be the prevention of antibiotic resistant bacteria infections through vaccination, a strategy that has already proven its great value to humanity (6). Several reasons suggest that vaccines would be a promising solution against AMR. First, antibiotics have shown to rapidly become obsolete and resistance emerges soon after their introduction, while vaccines allow long-lasting protection against infections and resistance has only rarely evolved after vaccination (13). Second, while antibiotics only hit a few metabolic target vaccines, based on the selected strategy, they can elicit a broad multi-target immune response reducing the probability of the evolution of resistant mutations. Furthermore, although major investments have been made to enrich antibiotic R&D pipelines, the discovery of innovative antimicrobial targets are running dry since the 1970s. Therefore, given the incredibly high pace with which pathogens are capable of developing resistance to new classes of antibiotics, focusing our attention exclusively on antibiotic R&D will not be sufficient (13, 45). In a marked contrast, thanks to incredible technological advancements of the last few decades, vaccine R&D pipelines are promising for the development of innovative and highly effective vaccines which can have an important contribution in controlling AMR (13, 18). Finally, antibiotics can only be used to treat individuals already infected, while successful vaccination campaigns can prevent the occurrence of infection, reducing the spread of the infectious agent and protecting the whole population through herd immunity (8, 20, 46). Vaccine evolution has allowed us to address several unmet medical needs and, given all of the reasons stated above, it should be considered a key solution in fighting emerging threats such as AMR (**Figure 1**).

CONCLUSIONS

Since their introduction, vaccines have helped save billions of lives all over the world. Empirical approaches were not sufficient to support the development of vaccines against pathogens for which no preventive strategies or treatments were available. Methodological and technological advancements have introduced the world to modern vaccinology approaches which have unlocked the possibility to develop novel vaccines against virtually any pathogen. The RSV and HIV case studies reported herein, are clear examples of how innovative technologies and their corollary applications have paved the way for new experimental approaches capable of tackling and possibly addressing these unmet global medical needs. Vaccines have provided the basis for a global and sustainable public health in the past and they can potentially continue to do so by addressing major and upcoming global threats like AMR.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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The Mononuclear Phagocytic System. Generation of Diversity

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We are living through an unprecedented accumulation of data on gene expression by macrophages, reflecting their origin, distribution, and localization within all organs of the body. While the extensive heterogeneity of the cells of the mononuclear phagocyte system is evident, the functional significance of their diversity remains incomplete, nor is the mechanism of diversification understood. In this essay we review some of the implications of what we know, and draw attention to issues to be clarified in further research, taking advantage of the powerful genetic, cellular, and molecular tools now available. Our thesis is that macrophage specialization and functions go far beyond immunobiology, while remaining an essential contributor to innate as well as adaptive immunity.

Keywords: mononuclear phagocyte, macrophage, tissue-specific function, monocyte, plasticity, macrophage heterogeneity, macrophage receptors

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INTRODUCTION

Participation in several Ceppellini workshops by one of the authors (SG) provided an opportunity to examine and present to young investigators some aspects of the unique features of the macrophage, a cell type with an ancient origin in eukaryotic evolution. SG's attachment to the macrophage family has extended over 50 years, rejuvenated over every decade as methodological advances brought new insights and information. However, their biological role in the multicellular organism has remained incomplete, eclipsed as accessory to the specific recognition, and effector functions of lymphoid cells. Metchnikoff already appreciated their professional phagocytic capacity, their digestive proficiency, and potential role in antimicrobial defense (1), while Ehrlich and Wright (2) drew attention to the role of antibodies and opsonins, which enhance phagocytic uptake by monocytes, macrophages, and polymorphonuclear neutrophils (PMN). The discovery of complement and, decades later, the plasma membrane receptors for the Fc domain of IgG specific antibodies and for C3 activated by the classical and alternative pathways, initiated pioneering studies by many investigators [reviewed by Taylor et al. (3)]. Hortega recognized the special properties of microglia in the Central Nervous System (CNS) (4). The discovery of Dendritic cells(DC) by Steinman and Cohn (5, 6), demonstrated their superior role in antigen capture, processing, and presentation to naïve lymphocytes of peptides, in association with the highly polymorphic Major Histocompatibility (MHC) antigens, thus inducing specific T and B lymphocyte activation and expansion (7). DC-like cells can be readily produced in culture of mouse bone marrow or human monocytes in Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF; CSF-2) and IL-4 (8). To some extent DC eclipsed the role of macrophages in adaptive immunity, although their role in innate immunity was secured by the discovery of Toll-like Receptors (TLR) (9). The discovery and characterization of cytokines produced by and acting

on macrophages, such as Tumor necrosis factor(TNF) (10) and IL-1 (11), prepared the way for anti-TNF therapy (12), to ameliorate destructive immunopathologies such as rheumatoid arthritis. Activation of macrophages by cellular immunity, characterized by Mackaness (13), was shown to be antigen dependent, but non-specific, and lead to the characterization of Interferon (IFN) gamma (14) as the sole mediator of classical activation produced by antigen-specific T lymphocytes and Natural Killer (NK) cells. After setting the stage above, further relevant milestones of macrophage history will be introduced in subsequent sections. Selected historic figures important in the present understanding of tissue macrophage diversity are shown in **Figure 1**.

THE MONONUCLEAR PHAGOCYTE SYSTEM, A DISPERSED ORGAN

Metchnikoff recognized migratory and sessile, fixed tissue phagocytic cells in his early studies of invertebrate development, by microscopy, and intravital labeling. Direct observation of their recruitment to foreign particles injected *in vivo* lead to further studies in many vertebrate species on their role in host defense against bacteria. Tissue macrophages were subsequently shown to be widely distributed as a system of related cells during development, in the adult steady state, during inflammation, and infection. Aschoff introduced the term Reticulo-Endothelial System (RES), hallmarked by the efficient clearance of particles from the circulation, and extravascular space (15). The imprecise RES nomenclature was replaced by that of the Mononuclear Phagocyte System (MPS) (16), to distinguish mononuclear monocytes and macrophages from PMN, while sharing their highly active capacity as phagocytes. Although widely used till the present day, this terminology is not perfect, since other cell types phagocytose dying cells, and some macrophage-related cells are poorly or even non-phagocytic (17). The diverse cells of the MPS cannot all be characterized by single antigen markers or unique functions expressed at all stages of cell differentiation or activation. Nevertheless, their origin and diversification have common features which point to the valid concept of a specific, dispersed myeloid lineage.

During mammalian development, macrophages derive from haematopoietic precursors in para-aortic regions of the embryo, the yolk sac and fetal liver, seeding organs such as the brain and other tissues before birth (18, 19). A paradigm shift over recent decades has shown that after birth, in the absence of inflammation, resident macrophages in adult tissues derive from embryonic macrophages which can persist, and gradually turn over locally throughout adult life (20–22). This is especially the case for microglia in the (CNS) and Langerhans cells in the epidermis. The bone marrow, which develops as the main haematopoietic organ perinatally, and fumctions throughout adult life (23), serves to replenish resident tissue macrophages, for example in the gut (24), where macrophages turn over more actively, and provides blood monocytes (25) in response to increased demand, for example during inflammation and infection (26). The chemokines and receptors which mediate distribution of monocytes and macrophages in the fetus and adult are not completely defined, nor the adhesion molecules which determine organ-specific localization. Chemokines of resident macrophages include fractalkine and its receptor, CX3CR1 (27), and inflammatory, and immune monocyte recruitment mediated by CCL2 and its receptor, CCR2 [**Figure 2**; (29, 30)]. Apart from these and related chemokines, recent studies have uncovered macrophage axonal guidance by semaphorins, and plexinA (31, 32). While resident macrophage populations, for example in the peritoneal cavity, persist locally, they can be induced by inflammation, to enter lymphatic vessels for delivery to lymph nodes (33), or to enter neighboring organs such as liver, by sterile local injury (34). Blood monocytes of bone marrow origin may remain inside the circulation and interact with the luminal surface of vascular endothelium (35), become part of sinus-lining endothelium, as Kupffer cells, or diapedese into tissues. Such recruited monocytes are transient in blood (24–48 h) and shorter lived (4–7 days) after migration into tissues, compared with resident macrophages of yolk sac origin e.g., microglia, which can be extremely long-lived. Other reservoirs of precursors and mature macrophages are found in splenic red pulp (36) or in secondary haematopoietic organs, such as liver.

While the dual origin of tissue resident macrophages is now widely accepted, there is still uncertainty about the relative contribution of the bone marrow in the adult steady state. In mouse liver, for example, early studies by van Furth and Cohn (37), before their embryonic origin was appreciated, argued for a major contribution of recently dividing bone marrow-derived blood monocytes to resident Kupffer cell populations. The pendulum has swung to yolk sac origin, perhaps too far, as acknowledged by more recent studies (38). The Geissmann group, investigating the origin of murine osteoclasts, showed that after initial perinatal formation of multinucleated cells in bone, monocytes of bone marrow origin are recruited and continue to fuse with osteoclasts throughout adult life (39).

GROWTH AND DIFFERENTIATION

Studies by Metcalf (**Figure 1**) on colony forming cells and lineage-specific growth factors contributed greatly to our understanding of haematopoietic stem cell growth and differentiation *in vitro* (40). Lineage tracing by several groups (41–43) built on studies by Stanley on CSF-1 [reviewed by Chitu and Stanley (44)] and on GM-CSF (45), the major

Abbreviations: ADGR, adhesion 7-transmembrane G-protein coupled receptor; BAI-1, brain-specific angiogenesis inhibitor-1; CSF1-R, macrophage colony stimulating factor receptor; DC, Dendritic cells; EMR, epidermal growth factor-like module-containing, mucin-like hormone receptor-like receptor; ITAM, immunoreceptor tyrosine-based activation motif; GM-CSF, granulocyte macrophage colony stimulating factor; GPCR, G-protein coupled receptor; IL, Interleukin; iPSC, induced pluripotent stem cell; MARCO, macrophage receptor with collagenous domain; MHC, major histocompatibility complex; MPS, Mononuclear phagocyte system; NK, natural killer cell; PD-1, Programmed cell death-1; PMN, polymorphonuclear leukocyte; RES, reticulendothelial system; scRNA seq, single cell ribonucleic acid sequencing; Sirp, signal regulatory protein alpha; SR-A, scavenger receptor A; Tie2, Tyrosine-protein kinase receptor for angiopoietins-2; TLR, toll-like receptor; TNF, tumor necrosis factor; TREM-2, Triggering receptor expressed on myeloid cells-2.

FIGURE 1 | Historic figures associated with macrophages, related cells and their specialized functions.

growth/differentiation factors for monocytes, macrophages, and DC. After initial description by von Kolliker in 1873 (**Figure 1**) (46), Loutit (47) produced proof of the bone marrow origin of osteoclasts; CSF-1 –deficient osteopetrotic op/op mice (48) lacked many, but not all tissue macrophage populations (49). Residual tissue macrophage populations such as microglia, for example, may depend on IL-34, a second ligand for the CSF-1 Receptor, since patients with profound human CSF-1 R deficiency have grossly abnormal CNS development attributed to the absence of microglia (50). Collin et al. have identified mutations which affect monocyte and DC growth and differentiation in humans; bone marrow transplantation and adoptive transfer of haematopoietic stem cells provide clinical and experimental models of monocytopoietic differentiation *in vivo* (51). Recent studies by Olsson et al. (52) and Yanez et al. (53) have demonstrated a binary origin of monocytes in the mouse, exploiting single cell and population RNA seq analysis and adoptive cell transfer.

In spite of these basic discoveries, we need more quantitative information on the number of monocytes, macrophages, and DC in human tissues, and their life span *in vivo*. Yona et al. traced the relationship of human monocytes in the steady state and the kinetic response of monocyte subpopulations to endotoxin administration *in vivo* (54). The subset of monocytic precursors which gives rise to osteoclasts remains to be determined; osteoclasts can be readily produced *in vitro* by culture of monocytes in CSF-1, and Rank Ligand (46), which should facilitate such studies.

TISSUE DISTRIBUTION AND ORGAN-SPECIFIC PROPERTIES

In the mouse, we have benefited from the availability of monoclonal antibody markers such as F4/80 to detect macrophages in the developing embryo, in the adult steady state and following a wide range of models of inflammation, infection, malignancy, and atherosclerosis. In addition, we used a panel of mab to identify tissue-specific heterogeneity of marker expression (3). **Figure 2** provides a schematic cartoon of these and additional macrophage plasma membrane receptors (55). With the aid of these reagents we identified substantial morphologic and antigenic heterogeneity of resident murine macrophages in different tissue environments such as CNS, spleen and bone marrow (28). Further studies demonstrated heterogeneous antigen expression of monocyte-derived macrophages in BCG-induced granulomata (56), as well as in multinucleated macrophage giant cells (57) and osteoclasts (58). Knowledge of the *in situ* phenotypes of human tissue macrophages is still limited.

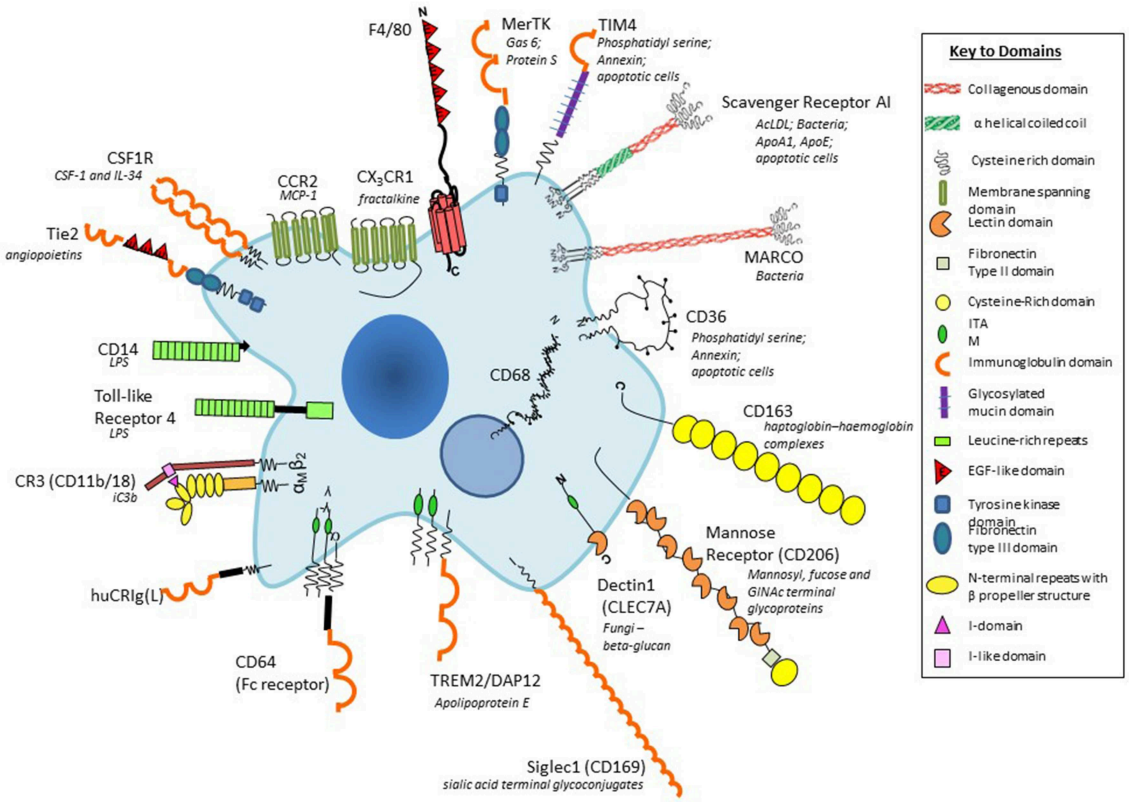

FIGURE 2 | Plasma membrane antigens and receptors expressed by macrophages. Macrophages are able to express a large repertoire of membrane receptors implicated in the recognition and uptake of foreign and modified self-ligands, some of which are illustrated here. These receptors incorporate a range of structural domains, illustrated schematically; they serve as useful marker antigens for immunocytochemistry and FACS analysis (e.g., F4/80, CD68, CSF-1 receptor, Mer-TK, CD64). They function as opsonic (antibody and or complement coated particles to enhance uptake via Fc and complement receptors) or non-opsonic, carbohydrate-binding lectins, and scavenger receptors. The phagocytic receptors mediate clearance of microbes (e.g., MARCO), apoptotic cells (for example CD36, SR-A, TIM4), and circulating ligands; CCR2, and CX3CR1 are examples of GPCR receptors for the monocyte/macrophage chemokines MCP-1 and fractalkine, respectively; other receptors bind growth promoting and regulatory cytokines, for example, CSF-1, and angiopoietins (Tie-2), and CD163 for clearance of injurious haptoglobin–hemoglobin complexes. Toll-like receptor-4 and CD14 react with bacterial membrane components such as lipopolysaccharide (LPS) to induce pro-inflammatory signaling; Dectin-1 recognizes fungi through beta glucan in their wall, activating a range of innate immunological responses. Siglec-1 (CD169), a receptor for sialic acid terminal glycoconjugates, mediates adhesion of host cells and microbes, whereas CD206, a receptor for clearance of Mannosyl-, fucose-, GlcNAc-terminal glycoproteins, is a prototypical marker of M2-type activation. The scavenger receptor SR-A internalizes polyanionic ligands such as modified lipoproteins, as well as selected microbes, whereas CD36 mediates adhesion and M2-induced macrophage fusion and giant cell formation. TREM-2 mutations have been implicated in neurodegeneration and osteoclast function. For further details, see BMC, with permission (28).

HETEROGENEITY OF TISSUE MACROPHAGES: ANTIGEN MARKERS

The F4/80 antigen(EMR1/ADGRE1), discovered by Austyn and Gordon (59), was used by Hume and others (60) to define monocytes, and macrophages in the mouse. F4/80 is mainly expressed on the plasma membrane, with minimal endocytosis, and is stable to aldehyde fixation; immunocytochemistry therefore can provide exquisite detail of plasma membrane processes in tissue macrophages, suggestive of potential interactions with neighboring cells. Regional variation in morphology and dendritic processes is particularly notable in the brain (61). F4/80, a member of a leukocyte 7-transmembrane, adhesion G protein-coupled receptor family, has been implicated in peripheral tolerance (62), but natural ligands have not been identified. It is also expressed by eosinophils in mouse and human; EMR1 has been identified in other species (63), but expression is transient in human monocyte-derived macrophages. A related molecule, EMR2 (CD312), discovered by Lin and Stacey (64), is expressed by human myeloid cells in blood and tissues, binds chondroitin sulfate B/dermatan sulfate and has been implicated in a human genetic syndrome, vibratory urticaria (65), associated with mast cell degranulation. EMR2 undergoes autoproteolytic cleavage of its extracellular domain to generate an N-terminal polypeptide agonist of GPCR activation.

The F4/80 antigen is expressed during mouse development from midgestation (19) and has been particularly useful in studies of microglia (61). It is also well-expressed in the adult mouse on resident tissue macrophages in the peritoneal cavity, red pulp of spleen, epidermal Langerhans cells, lamina propria

of the gut, and Kupffer cells; expression is low on alveolar macrophages in lung, and absent or minimal in white pulp and T-cell rich areas. F4/80 is absent on osteoclasts, metallophilic macrophages in the splenic marginal zone and on subcapsular sinus macrophages in lymph nodes, which express the pan-macrophage endo/lysosomal marker, CD68. Bone marrow-derived monocytes and tissue macrophages recruited to sites of inflammation, infection and malignancy in the mouse express F4/80 strongly.

SIGLEC-1(CD169, sialoadhesin)is a macrophage-specific sialic acid-recognition lectin discovered by Crocker on bone marrow stromal macrophages, at the center of haematopoietic islands (66). It is strongly expressed by marginal metallophils in mouse spleen and by subcapsular sinusoidal macrophages in lymph nodes. It has been implicated in retention and release of monocytes from bone marrow into the circulation. Other lectins widely expressed by macrophages, especially after alternative activation by IL-4/-13, include the macrophage mannose receptor (CD206) (67), and Dectin-1 (CLEC7A), identified as a receptor for fungal beta –glucan by Brown and Gordon (68) and Taylor et al. (3). Scavenger receptors implicated in clearance of apoptotic cells (69), non-opsonic microbial phagocytosis and lipoprotein endocytosis (70), include SRA-I/II, constitutively present on many tissue macrophages (71) and the structurally related collagenous receptor, MARCO (72), which is constitutively expressed by macrophages in the outer marginal zone of rodent spleen (73), but is induced on many tissue macrophages by microbial Toll-like receptor stimulation.

In addition to the above antigens, macrophages express plasma membrane receptors (28) involved in opsonic recognition of IgG antibodies (FcR), complement components (e.g., CD 11b/18), and other opsonins such as milk fat globulin. Other adhesion molecules include various integrins and CD44; plasma membrane receptors that mediate apoptotic cell clearance include an adhesion GPCR BAI-1 (17) and immunoreceptor tyrosine-based activation motif (ITAM) receptors, Tyro, Axl, and MerTK (74). Immunoregulatory receptors include TREM 1 and 2, SIRP alpha, and PD-1. These and receptors for growth factors, cytokines and chemokines have served as useful reagents for FACS, lineage and functional analysis, contributing to our knowledge of macrophage heterogeneity in mouse and human. CD11b expression, for example, is well-expressed on microglia and peritoneal macrophages whereas it is downregulated on alveolar macrophages and Kupffer cells *in situ*.

GENE EXPRESSION

Advances in analysis of macrophage mRNA expression by bulk and single cell sequencing have begun to provide a great deal of new information which has not yet been fully validated by protein expression *in situ* (75–77). However, important conclusions can already be drawn. These studies confirm that macrophages from different tissues are biosynthetically highly active, expressing a large number of diverse, yet canonical macrophage genes. However, tissue macrophages from different organs also express distinctive antigen and mRNA signatures (77) (**Figure 3**). Recent publications have reported scRNA-seq analysis of blood mononuclear cells (80), embryonic and adult cell populations, including human placenta (81, 82), which contains both fetal, and maternal macrophages. Improved methods of *in situ* protein expression (83, 84) are required to validate heterogeneity of genomic and epigenomic expression by macrophages isolated from different tissues. Spatial reconstruction of immune niches has been proposed by combining photoactivatable reporters and sc RNA-seq(NICHE-seq) (85). Consortia of investigators are contributing to a human tissue atlas (86), which has already lead to discovery of novel cell types and functions. Open access to data will extend knowledge of variation in gene expression by macrophages from different sources. This will illustrate developmental, physiologic, and pathologic expression and functions of resident and monocyte-derived macrophages, as well as indicating the cells with which they interact locally. Striking results have already been reported on the overriding effect of phagocytosis of apoptotic cells on gene expression by macrophages in different sites. These have used *in vivo* models in gut (87), for example, and include parabiotic experiments (88). The microbiome of the gut does not only affect the macrophage phenotype in its local microenvironment, but also systemically (89, 90), through release of microbial products.

POLARIZATION AND PLASTICITY OF MACROPHAGES

We used selected membrane markers to examine the phenotype of mouse peritoneal and human monocyte-derived macrophages in culture, following exposure to Th1 and−2 associated cytokines. In the mouse, IL-4, and subsequently IL-13, was shown to enhance expression and function of mannose receptors (CD206), whereas Interferon gamma selectively downregulated this marker (91). Since MHC class II expression was upregulated by both types of cytokine, we termed this process, alternative, and classical activation, respectively. The terminology M2 and M1 was introduced to include other prototypic stimuli such as immune complexes and macrophage expressed-signatures of selected marker genes (92, 93). We found, using a range of *in vitro* and *in vivo* models, that transglutaminase 2 expression, which is not specific for macrophages, was a consistent marker of alternative macrophage activation in humans and mice (94). Subsequent studies by many investigators showed that macrophage polarization involved a spectrum of changes in gene expression (95); to be a useful concept, we proposed that the term alternative activation should be restricted to the prototypical Th-2 cytokines, IL-4, and IL-13 and their common and specific plasma membrane receptors (96). Microarray analysis of macrophage populations using a range of activation and regulatory stimuli, indicates that modules of genes can be identified as signatures to distinguish among different forms of activation. Further analyses of single cell RNA, and protein expression of gene signatures by yolk sac- and bone marrow-derived macrophages and their correlation with distinct functions such as cytotoxicity, and tissue repair, are required to refine polarization in individual organs.

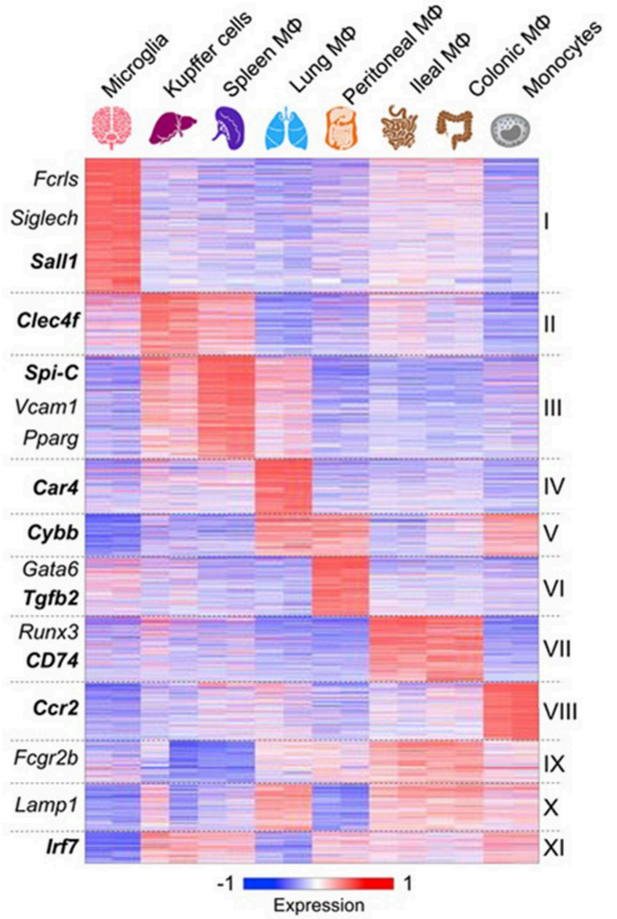

FIGURE 3 | Macrophages express canonical and tissue-specific mRNA signatures. From (77) for further details, with permission. See also (78), ImmGen Consortium (79).

Both classical and alternative macrophage activation can be divided into two distinct phases, an initial priming step by the appropriate cytokine, and completion by a phagocytic or microbial stimulus which induces further changes in gene expression and serves to localize macrophage effector activity. Microbial uptake enhances cytotoxic and pro-inflammatory activity of interferon-primed, classically activated macrophages, whereas uptake of apoptotic cells by IL-4 treated macrophages, enhances anti-inflammatory gene expression by alternatively primed macrophages (97). In experimental models, LPS can induce paradoxical enhancement of JNK activation following Scavenger receptor ligation of IL-4-primed macrophages, suggesting that the outcome will depend on the nature of the phagocytic receptor involved (98).

Priming of macrophages can also induce an adaptive enhancement of microbial phagocytosis and innate immune function. For example, LPS or microbial stimulation upregulates MARCO expression enabling subsequent enhanced uptake of Neisseria meningitidis via this receptor (99, 100). This observation harks back to the earlier studies of Mackaness on macrophage activation by BCG and Listeria monocytogenes, shown to be antigen dependent, but non-specific for the inducing organism (13). Netea et al. have extended this phenomenon, an example of "trained immunity" (101, 102), and have implicated epigenetic mechanisms in its imprinting.

These concepts are important in attempts to reverse polarization, for example of tumor associated macrophages, for potential immunotherapy. Evidence that the macrophage phenotype *in vivo* is plastic and reversible by adoptive transfer to different tissue microenvironments is sketchy. Van de Laar et al. have shown that yolk sac macrophages, fetal liver and adult monocytes efficiently colonize the empty alveolar niche of *Csf2rb*$^{-/-}$ mice, unlike mature liver peritoneal or colon macrophages (103). We have found that once macrophages have differentiated terminally, for example to a resident peritoneal phenotype, they cannot be induced to express adhesion receptors characteristic of other terminally differentiated macrophages such as those found in bone marrow haemopoietic clusters. Furthermore, experiments need to distinguish between changes in cell populations and individual cells. However, the phenomenon of induced pluripotency (104) indicates that transcription factors and chromatin conformation can enable true plasticity and the ability to give rise to embryonic stem cells, able to generate different somatic cell types, including macrophages (105) and microglia (106) *de novo*.

GENERATION OF DIVERSITY IN TISSUE MACROPHAGES

The evidence that resident embryo or bone marrow-derived populations of tissue macrophages, distributed throughout organs in the steady state, acquire distinct phenotypes as well as expressing core macrophage properties, raises a fascinating problem of origin of their diversity. The extent of adaptation by monocytes recruited by infection to different tissue environments, for example in granuloma formation, requires further characterization. In order to establish a testable hypothesis to account for the generation of diversity, we have to keep in mind several properties which distinguish macrophages from T and B lymphocytes, in which antigen receptor gene rearrangement and clonal selection have provided unexpected solutions to account for repertoire diversity and antigen specificity. Macrophages express a broader range of receptors than lymphocytes to distinguish foreign, modified-self and self-ligands; these include proteins and peptides, carbohydrates, nucleic acids, and lipids. Macrophage receptors can be viewed as "hard wired," unlike the more selective, antigen-specific receptors of adaptive lymphocytes. Tissue macrophages are terminally differentiated, capable of only a limited degree of proliferative capacity once they enter tissues. Clonal selection can therefore be ruled out. We do not know the size of the macrophage repertoire, but it must be substantial to accommodate interactions with other cell types within the body, including macrophages themselves, as well as so-called "pattern recognition receptors" for exogenous and endogenous ligands. Many investigators acknowledge that the local tissue as well as

exogenous micro-environment must play a specifying role in inducing or selecting expression of a particular constellation of surface receptors and gene products [for example (75, 107)]. In addition, macrophages can recognize a host of intracellular ligands in their cytosolic, biosynthetic, secretory, or endocytic compartments. However, chromatin conformation, transcription factors and enhancers, in addition to epigenetic mechanisms, must also determine the programme of differential gene expression, and modulation of the macrophage phenotype (108–112). T'Jonck et al. have discussed the role of niche signals and transcription factors involved in tissue resident macrophage development in detail (113).

These considerations leave many questions as to how, when and where, and specifically by which intrinsic and environmental mechanisms, diversity is achieved. Surprisingly little consideration has been given to the nature of the diverse ligands in the extracellular matrix of different tissues (114); nor the role of various epithelia, endothelia, mesenchymal, and neuro-endocrine cells, all of which interact with macrophages as a result of their unique migration and organ distribution (83, 84, 115, 116).

TISSUE-SPECIFIC FUNCTIONS OF MACROPHAGES

Tissue macrophages express general, prototypic, functions throughout the body which contribute to homeostasis, recognition and responses to intrinsic and external perturbation, restoring physiologic stability, and contributing to repair after injury. In different organs they adapt to different micro-environments with variations on the themes of clearance of particles and soluble ligands, digestion or storage in lysosomes, constitutive, and induced biosynthesis, and secretion. They interact with living or dying cells and microbes, blood and lymph, undergoing metabolic adaptation, and altering adhesion to extracellular matrix as they migrate, through different locations over time. In the process, they may respond to injurious stimuli by autophagy, cell growth or death. Nevertheless, we can already discern remarkable variations in organ-specific functions to which they contribute; these include a central role in haematopoietic turnover, and haem degradation (117, 118), lymphoid trafficking of immune cells (33); mucosal physiology, for instance in the gut (119, 120); remodeling in the CNS (107, 121, 122); neural- adipose tissue metabolism (123), and adipose- sympathetic nervous interactions (124); and electrical activity in the heart (125). Current studies in single cell RNA and protein expression by tissue macrophages will provide more examples of trophic and defense functions, contributing to embryonic development, anatomic, physiologic, and pathologic processes. Returning to our earlier discussion of how such diversity might be generated, it seems likely that encounters with different ligands in their tissue microenvironment can exploit pre-existing or induce novel sensors to activate adaptive changes in transcription and epigenetic modification; this begs the question of the extent and mechanisms of initial tissue-specific receptor diversification. While differentiation can generate a core panel of recognition molecules on and within the macrophage, it may be necessary to postulate further induction, feedback amplification, or selection by as yet unknown somatic gene expression mechanisms. Investigating the details of osteoclast and DC development *in vivo* and *in vitro* may provide further clues to novel molecular mechanisms.

CONCLUSIONS

Recent progress in molecular and cellular biology have brought exciting insights into view, enabling us to characterize monocyte/macrophage heterogeneity *in situ*. Understanding the themes of their functions within multicellular organisms across a range of evolutionary stages will make it possible to discover a unifying pattern extending far beyond innate or adaptive, cellular and humoral immunity. The challenge will be to imagine the properties underlying the genes and molecules which can lead us to such knowledge. Finally, we need to consider the implications of monocyte/macrophage heterogeneity for therapy. Factors to be taken into account for macrophage-directed immunotherapy include the expression of target antigens on distinct subpopulations, the route of administration, risk of off-target effects and species differences. Similarly, for potential adoptive cell therapy, the origin, differentiation, proliferative capacity and activation status have to be defined, as well as genetic compatibility.

AUTHOR CONTRIBUTIONS

SG conceived and wrote the manuscript. AP reviewed and edited the manuscript and designed the figures.

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OPINION
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Sharing Knowledge With Young and Established Students of Immunology by the Neapolitan Gulf at the Ruggero Ceppellini Advanced School

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INTRODUCTION

In his *Origin*, Charles Darwin led the foundations to debunk the long-held belief that man and animals derive from separate lineages, landing the final blow in *The Descent of Man*. The discovery in the mid-1980's that fertilized mammal eggs must have male components to generate healthy offspring had similarly dramatic consequences on other religious beliefs, as discussed in "*Genetics: immaculate misconception*" (1). In the Catholic calendar, the 8th of December is dedicated to the Virgin Mary. The occasion was celebrated with loud fireworks cracking during the second night of the 25th course of the EFIS-EJI Ruggero Ceppellini Advanced School of Immunology, held at Castellammare di Stabia, near Naples, 7th−9th December 2014. A faculty of 13 gathered together with 60 attendants from 19 countries to discuss the theme *Maternal Immune System in Pregnancy*. While the conclusions of the course were not quite as dramatic as Darwin's and Surani's, new exciting concepts were discussed that had already emerged at a previous meeting held in Cambridge in 2013 to celebrate the 60th anniversary of Peter Medawar's famous article on the "immunological paradox" of pregnancy (2). I had the honor of directing both events, together with Ashley Moffett, and learned a great deal.

This brief article is a report on the activities during that 25th course, as well as an opportunity to celebrate the importance of the Ceppellini School to connect young immunologists with leader scientists in their fields, as well as to spur new collaborations. With the generous support of the EFIS-EJI, the Bill and Melinda Gates Foundation, and the International Union of Immunological Societies, a record number of travel fellowships was offered to 13 participants from African countries, including South Africa, Kenya, Nigeria, Gabon, and Cameroon. This was appropriate because it is in Sub-Saharan Africa (SSA) that maternal morbidity and mortality is highest due to pregnancy complications, such as the hypertensive disorder of pregnancy pre-eclampsia, still birth or intrauterine growth restriction (3).

ACTIVITIES DURING THE 2014 COURSE

On the first day of the course, Silvia Fontana Zappacosta talked about the ethos and history of the School founded by her late husband Serafino Zappacosta. One of the remits of the School is to "foster wider interest for immunology and to attract to the discipline young scientists, also from disadvantaged countries" (4). I introduced the course with a brief synopsis of each lecturer's topic and told the story of my own connection to the Ceppellini School. My late maternal uncle Tommaso (Tommi) Meo trained with Ceppellini himself in the 60's and 70's in Turin

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and Basel. Ceppellini made seminal contributions for the advancement of our understanding of immunogenetics (5). Among the factors determining pregnancy outcome are immune system genes—that is combinations of certain variants of genes coding for Human Leukocyte Antigens (HLA) and Killer-cell Immunoglobulin-like Receptors (KIR) (6). How odd that part of my research today was the subject of my uncle's science with Ceppellini and that he so excitedly narrated to us on his summer visits back in our native Southern Italy. John Trowsdale (University of Cambridge) reviewed the "ABC" of KIR and HLA, explaining how the system may have evolved to deploy the two A and B haplotypes that code for KIR receptors on Natural Killer (NK) cells to bind HLA-C on fetal trophoblast cells. Ashley Moffett (University of Cambridge) discussed how the KIR and HLA systems may have evolved and can be used to study population history (7). Annettee Nakimuli (Makerere University and Mulago Hospital, Kampala, Uganda) discussed the diverse KIR and HLA genes that cause susceptibility to or protection from pregnancy disorders in Europeans and Africans (8). Allison Elliott (London School of Hygiene and tropical Medicine and Uganda Virus Research Institute, Entebbe, Uganda) presented fascinating data on the impact of helminth infection during pregnancy and the outcomes in the offspring. Angela Santoni (University of Rome La Sapienza) reviewed leukocyte trafficking and the changes occurring during pregnancy. On the second day, Elizabeth Simpson (Imperial College, London, UK) gave a historical background on multiple histocompatibility antigens and how the maternal immune system is aware of fetal antigens yet does not mount an immune response against the fetus. Tamara Tilburgs (Harvard University, Cambridge, US and now at the Cincinnati Children's Hospital, US) discussed the delicate balance that the maternal immune system must strike between fetal tolerance and antiviral immunity. Jakob Michaelsson (Karolinksa Institut, Stockholm, Sweden) reminded the audience that the fetus also has its immune system that may engage with maternal antigens, with consequences on micro-chimerism. Marise Alegre (University of Chicago, US) revised the evidence that tolerance can be induced experimentally to transplants. Anthony De Tomaso (University of California Santa Barbara, US) talked about the strange and fascinating life of a basal chordate that uses allorecognition to regulate stem cell parasitism. In the third and last day, Ennio Carbone (Karolinksa Institute, Stockholm, Sweden and University of Catanzaro, Italy) opened the lectures with an overview on tumor immunology. Tom Gajewski (University of Chicago, US) followed up highlighting the immune pathways in the tumor microenvironment that may be operating also at the maternal-fetal interface, with the engagement of several inhibitory checkpoints. The course ended with my closing lecture on mouse models of immunogenetics of pregnancy.

In the typical spirit of the Ceppellini School, the presentations were enriched by ample discussions and debates in which both faculty and students participated actively. The search for elusive pathogenic T cells in pregnancy complications was discussed as it was the antigen specificity of these effector T cells, which most likely are HLA-C-restricted. Another theme was the importance of studying human populations in which the prevalence of pregnancy complications is highest. New technology that can help visualize lymphocytes at the maternal-fetal interface were discussed, including imaging approaches. Finally, various routes of vertical transmission were considered, including through maternal monocytes and fetal placental macrophages (i.e., Hofbauer cells).

FOSTERING YOUNG IMMUNOLOGISTS AND FACILITATING COLLABORATIONS

There were plenty of opportunities for the participants to interact among each other and with the faculty members over lunches, coffee breaks, and the poster session. Several collaborations stemmed from this course and continue till today. Both Anthony De Tomaso and Allison Elliott, two of the members of the faculty at this course came to spend a year as visiting Fellows of King's College, Cambridge, where myself and Ashley Moffett are also Fellows. Annette Nakimuli and Ashley Moffett have strengthened their collaboration and have since initiated a series of initiatives within the Cambridge-Africa partnership to improve patients care in the Department of Obstetrics and Gynecology at the Makerere University, including several trips from Cambridge obstetricians to visit Uganda. For example, Catherine Aiken, also at our Department, now mentors Imelda Namagembe's PhD thesis in Uganda, that focuses on improving maternal health.

Despite not present at the course, Stephen Tukwasibwe, then a research assistant in the same hospital of faculty member Annettee Nakimuli, became interested in the immunogenetics of pregnancy. Having worked successfully on the genetics of resistance to malaria and secured a Wellcome Trust PhD grant, Stephen started his thesis at Makerere University under the supervision of Annettee Nakimuli and my co-mentorship, to test the hypothesis that *Plasmodium* may have selected for those genetic variants that may protect from malaria but expose women to pregnancy complications in SSA. Stephen has since visited Cambridge several times working at the Pathology Department as part of his thesis. One of the participants, Iva Filipovic from Serbia, was completing her MSc degree at Imperial College, London, during the course and was very keen to learn more on immunology of pregnancy. She secured a PhD Studentship from the University of Cambridge Center for Trophoblast Research and came to work on her PhD as a graduate student of King's College and in my laboratory to study the gene expression profile of innate lymphoid cells in the uterus of mice (9). She is currently working as a post-doc at the Karolinska Institute and I look forward to seeing her future successes.

NEW CONCEPTS AND RECENT PROGRESS IN THE FIELD

Peter Medawar in 1953 famously proposed three mechanisms underlying placental tolerance: (i) anatomical separation of mother and fetus; (ii) antigenic immaturity of the fetus; (iii) immunological unresponsiveness of the mother. Bearing in mind these proposals were formulated in light of the progress made during those days in transplantation immunology, and with

unimaginable less knowledge of the details of the human immune system then we have today, it is perhaps not surprising that none of these three mechanisms have been fully substantiated—although they have influenced generations of immunologists of reproduction. On the contrary, we know that the placenta is not such a tight barrier and cells can mix in both directions. We also know that the fetus is not antigenically immature and the mother is not unresponsive. Indeed, pregnant women can make both T cells and antibodies that recognize fetal antigens (e.g., anti-D antibodies in Rhesus incompatibility).

One major conceptual shift in the immunology of pregnancy is the understanding that pregnant women are not immunosuppressed. Changes in the immune system during pregnancy may however be responsible for the greater morbidity and mortality of mothers and infants infected with certain pathogens (10). The emergence of new epidemics has attracted the attention of investigators who are now addressing the mechanisms of vertical transmission of certain pathogens, e.g., Zika virus (11, 12). That microbes are integral part of human health and disease has become established in the recent past, perhaps best illustrated by the influence of the gut microbiota on the immunotherapy of cancer (13)—one of themes of the 2019 course (*Microbes, Immunity and Cancer*) of the Ceppellini School (14). Transplantation immunology also may be influenced by microbes (15, 16), however the search for a placental microbiome has so far been elusive (17). Yet, maternal infections may have repercussions on neuropsychiatric disorders (18) and the development of the immune system in the offspring. Clinical trials are ongoing to evaluate the effectiveness of vaccinating mothers to prevent children's allergies (19, 20).

There are obvious selective disadvantages in a strategy that would suppress the immune system of pregnant women to allow the implantation and growth of the placenta. The placenta evolved much later than the immune system and it is reasonable to think that placentation and immunity have co-evolved agreeably, rather than embarking in a deleterious conflict. One illustrative example may be the interactions of maternal KIR on uterine NK cells with fetal HLA-C molecules on the placental cells, which may engage in a molecular conversation that, rather than leading to allorecognition-driven rejection, may in fact contribute to uterine vascular remodeling and placental growth (6). Adding to the complexity of the maternal-fetal interactions is the heterogeneity of immune cells, revealed recently by singe-cell RNA-sequencing (21) and mass cytometry (22). Mass cytometry has been applied to study also the fluctuations in blood immune cells throughout pregnancy (23, 24). Multiple populations of innate lymphoid cells (9, 21, 25), regulatory T cells (26), and macrophages (27) compose the diverse immune cell landscape operating at the maternal-fetal interface, which varies during the stages of pregnancy and it is therefore difficult to decipher precisely. New technology such as three-dimensional organoid cell cultures (28) may help to determine some of the mechanisms underlying placentation (29). Advances in typing polymorphic KIR and HLA genes (30, 31) may also help to shed light on the immunogenetics of pregnancy. Although the interactions of maternal KIR with fetal HLA-C may be a pivotal one to activate uterine NK cells and determine the outcome of pregnancy (6), the importance of the interaction of NK cell receptors with self HLA class I molecules is emerging, in a process known as NK-cell education. We have shown recently that NK-cell education in the uterus may follow different rules than in the blood (32) and that NK-cell education reduces the risk of pregnancy complications in women genetically programmed to engage the inhibitory NKG2A receptor on NK cells (33). The next grand challenge is to precisely decipher the multiple and changing interactions between mother and fetus in the decidua, to eventually manipulate them in order to improve the outcome of pregnancy (29).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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MINI REVIEW
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Recirculation and Residency of T Cells and Tregs: Lessons Learnt in Anacapri

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"Location, location, and location": according to this mantra, the place where living beings settle has a key impact on the success of their activities; in turn, the living beings can, in many ways, modify their environment. This idea has now become more and more true for T cells. The ability of T cells to recirculate throughout blood or lymph, or to stably reside in certain tissues, turned out to determine immunity to pathogens, and tumors. If location matters also for human beings, the inspiring environment of Capri Island has contributed to the success of the EFIS-EJI Ruggero Ceppellini Advanced School of Immunology focused on "T cell memory," held in Anacapri from October 12, 2018 to October 15, 2018. In this minireview, we would like to highlight some novel concepts about T cell migration and residency and discuss their implications in relation to recent advances in the field, including the mechanisms regulating compartmentalization and cell cycle entry of T cells during activation, the role of mitochondrial metabolism in T cell movement, and the residency of regulatory T cells.

Keywords: T cells, Tregs, cell migration, cell cycle, recirculation

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INTRODUCTION

This minireview is inspired by the EFIS-EJI Ruggero Ceppellini Advanced School of Immunology about "T cell memory" 2018 (1) and will expand in further detail two hot topics discussed during the course: T cell migration and residency.

T cell differentiation and function are strictly related to their distribution within different lymphoid and non-lymphoid compartments. In physiological conditions, naive T cells recirculate through secondary lymphoid organs (SLOs), increasing the opportunity to encounter the antigen. After infection, vaccination, or tumor growth, the draining lymphoid compartments undergo dramatic changes, promoting naive T cells' interaction with antigen-presenting cells and subsequent T cell activation. Activated T cells undergo a strong proliferation (so-called clonal expansion) and deep changes in their metabolism (2, 3). The process culminates with T cell differentiation and the generation of short-lived effectors and long-lived memory cells (4–6). Effector T cells migrate broadly, reaching the site of infection or tumor growth where they exert their effector functions before dying. Memory cells persist in the body, circulating between blood and lymphoid or non-lymphoid tissues as conventional memory T cells, or residing in peripheral tissues as resident memory T cells (Trm) (7). Trm represent a first-line defense against tissue

damage and pathogen invasion (8, 9). However, the functional distinction between Trm and conventional effector/memory T cells needs to be clarified. Moreover, it is now clear that some technical caveats may hinder an appropriate and complete analysis of these cells (10). A better understanding of the immunological and metabolic signals dictating the switch between T cell recirculation and residency is needed. Here, we will focus on some emerging concepts regarding this topic: first, the relation between the cell cycle phase and migration during T cell activation; second, the role of mitochondria relocation for T cell movements and compartmentalization; finally, the features of residency of a well-known tissue-infiltrating T cell population, i.e., the regulatory T cells (Tregs).

T CELL RECIRCULATION AND CELL CYCLE

After development in the thymus, naive T cells reach the blood circulation, and continuously circulate between blood and SLOs. This journey is finely regulated by the expression of specific homing molecules. Indeed, the L-selectin CD62L expressed by naive T cells mediates their entry into lymph nodes (LNs) by binding ligands expressed on high endothelial venules (HEVs). This binding overcomes blood shear forces, leading to T cell rolling on HEVs (11). At this stage, the interaction between the CC chemokine ligand 21 (CCL21) expressed on HEVs and the CC chemokine receptor 7 (CCR7) on T cells activates the integrin lymphocyte function-associated antigen 1 (LFA1). Activated LFA1 binds the intracellular adhesion molecule 1 (ICAM-1), mediating T cell arrest on the endothelium. Consequently, T cells migrate across the blood vessels and enter the tissue (12). Once in the LN, naive T cells are guided in the paracortical region, also known as T cell zone. In this area, naive T cells interact with dendritic cells (DCs), scanning for the presence of the cognate antigen. It has been estimated that one DC can be scanned simultaneously by up to 500 naive T cells (13, 14). Migration in this area is regulated by a gradient of chemokines and local factors. The chemokine CCL19, produced within the T cell zone, increases T cell motility and promotes T cell–DC interactions by binding CCR7 on the T cell surface (15). Furthermore, after immunization, naive CD8 T cells upregulate CCR5, which binds CCL3 and CCL4 produced at the site of the CD4 T cell–DC interaction in the immunogen-draining LNs (16).

Hence, migration in the SLOs seems to be not only a stochastic process but rather a finely regulated mechanism which increases the probability of antigen recognition. In the case that this rare event occurs, T cells undergo a series of dramatic changes. Resting naive T cells are activated by the integration of three signals: antigen recognition (signal 1), co-stimulation (signal 2), and cytokines, released at the site of T cell–DC interaction (signal 3) (17). This process culminates with the extensive proliferation of antigen-specific T cells, named clonal expansion. T cell expansion is driven by T cell–DC interaction within specialized niches in SLOs and is controlled by several factors which promote the rapid entry of T cell in the cell cycle (18–20). The final goal of this process is to increase the number of T cells capable of eliminating the antigen. It has been estimated that, in the first week of a typical primary T cell response, CD8 T cells can increase their number to about 100 times or more (21). At this point, deregulation of the cell cycle could deeply affect the ability to develop a proper T cell response. For example, a reduced clonal expansion could lead to a decreased number of effector and memory T cells, with consequent loss of protection. Furthermore, it has been hypothesized that the inability to mount an effective primary T cell response in old age and the vaccination failure occurring in elderly persons could be correlated with defects of T cell clonal expansion (22, 23).

Expanding T cells modulate the expression of homing molecules, preparing themselves to reach the peripheral tissue, the site of antigen entry. Retention in SLOs is controlled by the sphingosine-1-phosphate (S1P) receptor expression on T cells. S1P is a lipid molecule that is more concentrated in the blood and in the lymph than in tissues (24). S1P receptor expression is increased in naive T cells, leading to egress from SLOs. Activated T cells upregulate CD69, which prevents S1P receptor expression, holding T cells in the SLOs until the completion of differentiation into effector cells, which can take a few days (25). Once completely differentiated, effector T cells downregulate CD69, and migrate along the S1P gradient. Effector T cells also downregulate CD62L and express chemokine receptors that guide them to the site of infection (26).

The kinetic of expansion and migration is poorly defined. Indeed, although it is known that clonal expansion starts in SLOs, the location where activated T cells progress and/or complete their cell cycle is still unclear. To date, the few tools available for the analysis of dividing antigen-specific CD8 T cells, such as cell-labeling dyes and anti-Ki67 antibody, show some important limitations. Indeed, cell-labeling dyes do not allow evaluating whether cells found in one organ proliferated locally or rather migrated in this organ after division (19, 27). Ki67 is a nuclear protein expressed by cells in all the phases of the cell cycle (G1, S, G2, and M), except for those in G0 (or quiescent). Hence, Ki67 analysis alone does not distinguish proliferating cells (in S-G2-M) from those in G1, which may remain for a long time in G1, or even revert to G0 (or quiescent) without dividing (28, 29). We recently set up a new flow cytometric method for the cell cycle analysis of CD8 T cells, which was based on the combination of Ki67 expression and DNA content analyses and allowed us to discriminate between cells in the G0, G1, and S-G2/M phases. By using this method together with a novel gating strategy for the analysis of actively responding T cells, we demonstrated that, at early times after vaccination in mice, cycling antigen-specific CD8 T cells (cells in the S-G2-M phases) were present in the blood, which is usually not considered a site of proliferation (**Figure 1**) (30). This finding questions the general view by which activated T cells proliferate locally in SLOs and only after completing their cell cycle and differentiation enter the blood circulation, reaching the infection site. In addition, studies on cancer patients have shown that antitumor CD8 T cells increase Ki67 expression after checkpoint inhibitor treatment, suggesting that unleashed T cells can actively cycle in the blood after therapy (31, 32).

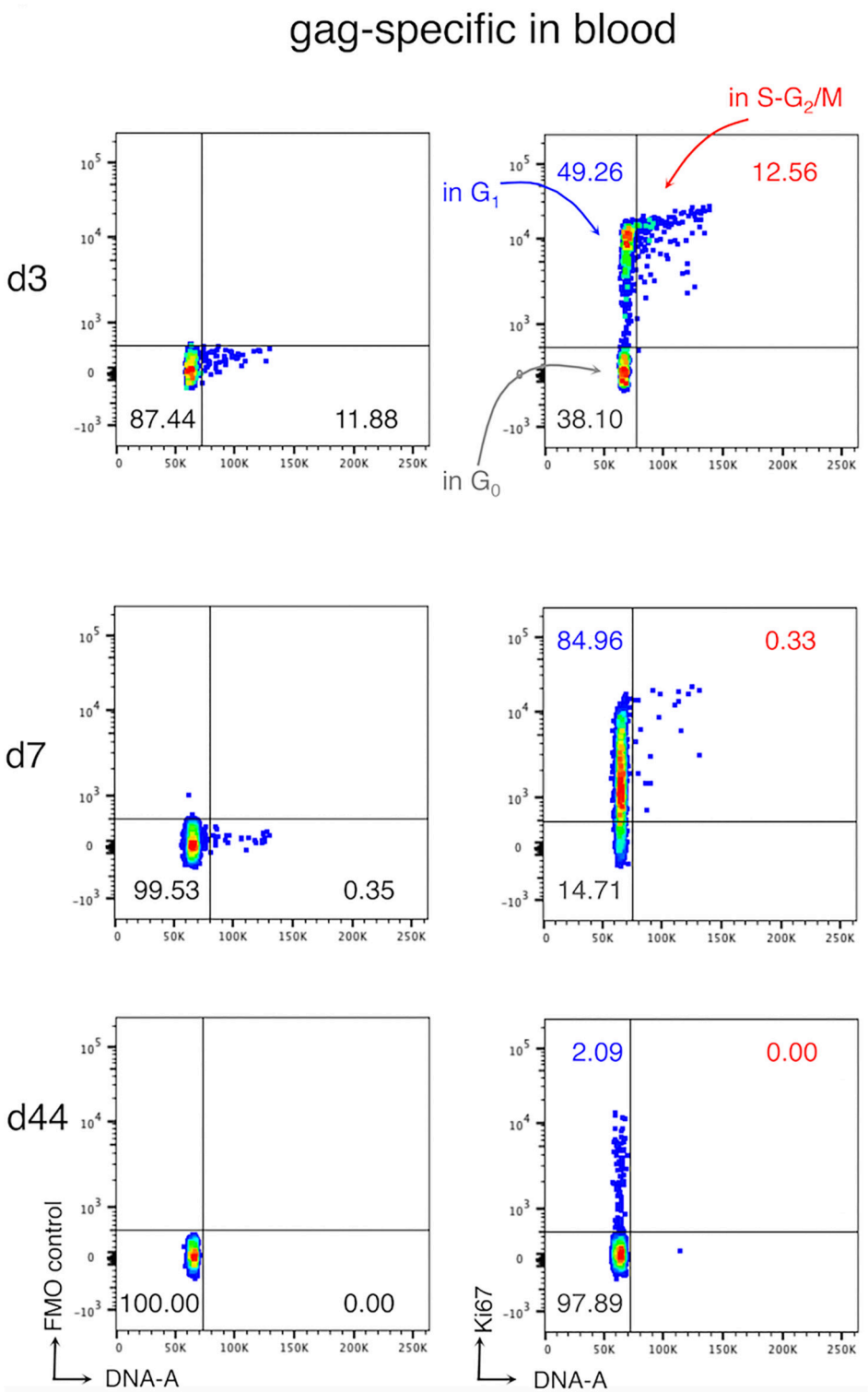

FIGURE 1 | Cell cycle analysis of antigen-specific CD8 T cells in the blood after vaccination. Female Balb/c mice were primed and boosted with viral vectors expressing the model antigen gag of HIV-1. At days (*d*) 3, 7, and 44, post-boost blood was collected and blood cells were analyzed with our new method. The figure shows a typical ki67/DNA staining profile of gag-specific CD8 T cells in the blood. Fluorescence Minus One (FMO) controls (*left*) and Ki67 staining (*right*) are shown, as indicated; the *numbers* represent the percentages of cells in the corresponding quadrant. Figure adapted from (30).

MITOCHONDRIAL DYNAMICS IN MEMORY T CELLS AND T CELL MIGRATION

In the past, immunologists did not take seriously into account T cell mitochondria since they are poorly represented within a T cell, and T cells are mainly considered as relying on glycolysis for their principal functions. In recent decades, a large body of evidence emerged on the crucial role that the mitochondria, their metabolism, and their morphological dynamics have on these cells. Nowadays, the pivotal role of mitochondrial morphology changes in almost all processes that are essential for a correct T cell development and function is clear and evident (33). Thus, these less attractive organelles suddenly became "main characters" for several immunologists in recent years.

Mitochondria, the cellular energetic hubs, are highly motile organelles, continuously fusing and fragmenting (a.k.a. fission) their network under the control of the so-called mitochondria-shaping proteins (34) (**Figure 2**). Drp1 and Dyn2 are the main players controlling fission in concert (35), while mitofusins 1 and 2 and Opa1 are the principal proteins orchestrating mitochondria fusion (36, 37). The balance between these opposing events, at every time or cell demand, determines organelle morphology, which acts as an intracellular signal that instructs different metabolic pathways, reflecting the different physiological functions of the cell. For instance, an elongated network sustains oxidative phosphorylation (OXPHOS) for a correct assembly of the electron transport chain (ETC) complexes, and an optimal ATP production, besides diluting the matrix content (38). A fragmented network, instead, promotes aerobic glycolysis and mitophagy or accelerates cell proliferation in response to nutrient excess and cellular dysfunction (38). Mitochondrial morphology directly regulates T cell differentiation *in vitro* by affecting the engagement of these alternative metabolic routes upon activation. Mitochondrial fusion-dependent fatty acid oxidation with a predominance of OXPHOS is a hallmark of a memory cell signature, while an effector cell subtype mostly relies on fission-dependent glycolysis (39, 40). Thus, mitochondrial dynamics controls T cell fate. Evidence *in vivo* of these findings, together with the molecular mechanisms explaining how mitochondrial dynamics can orchestrate these metabolic shifts and T cell fate, came soon after. Indeed, our lab showed that mitochondrial fragmentation, favoring glycolysis in effector T cells, is dependent on the Erk1-mediated activation of Drp1. Further and interestingly, an additional—but not mutually exclusive—transcriptional mechanism sustains the metabolic shifts in T cell differentiation. Upon T cell receptor (TCR) engagement, in T cells with an elongated mitochondria, the extracellular calcium uptake is exacerbated [presumably because of an inability of the un-fragmented mitochondria to reach the immunological synapse and to buffer calcium (41)], this leading to alterations on the mTOR–cMyc axis, decrease of cMyc expression, and related defective transcription of glycolytic enzymes, cMyc being known as a promoting factor in the transcription of glycolytic enzymes upon T cell activation (42). The consequence is a prominent oxidative metabolism and a memory-like phenotype for these T cells (43). Thus, in sum, memory T cell differentiation is driven by ERK1- and cMyc-dependent mitochondria morphological changes.

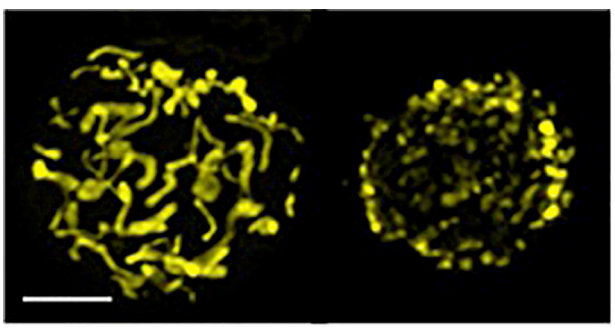

FIGURE 2 | Elongated and fragmented mitochondria morphology in T cells. Confocal z-stack acquisition and 2D reconstruction of an elongated (*left*) or fragmented (*fissed*, *right*) mitochondrial network of Jurkat single cells transfected with mtYFP (*scale bar*, 5 μm). Picture modified from (34).

More interestingly, for this review's purpose, the capability of memory T cells to reach the tissues and being resident, rather than to recirculate in the periphery, crucially relies on the ability of these cells to (trans)migrate and extravasate into and from the blood vessels. These basic processes also strictly depend on mitochondrial dynamics. Polarized T cells need to accumulate their mitochondria at the uropod during migration, to fuel the ATP-consuming myosin II cell motor. Drp1-dependent fragmentation of the mitochondria is essential to allow this organelle relocation, while unbalancing the morphology toward an elongated mitochondrial network strongly impairs T cell chemotaxis (44). *In vivo* extravasation and invasion of T cells are regulated likewise. During their trans-migration across an endothelial layer, lymphocytes squeeze and insert their nuclei into a subendothelial pseudopodium (45), a process heavily relying on the activity of the myosin motor (46) and requiring Drp1-dependent mitochondria fragmentation (43). Consistently, *in vivo* Drp1 removal from T cells inhibits their extravasation from the blood toward SLOs, and toward "danger sites" (43).

Noteworthy is that Drp1 knockout (KO) T cells are deficient in cell migration, even though their metabolism is shifted toward an OXPHOS-based metabolism, ideally producing more ATP to fuel the myosin II, which should drive a higher migration rate. This apparent paradox underlines the cell's need to better modulate the relocation of the mitochondria for a local, subcellular production of mitochondrial ATP rather than for a general mitochondria functionality.

Overall, these findings shed light on a new and crucial role for mitochondrial dynamics in T cell differentiation and function, paving the way for new, and important therapeutic opportunities through pharmacological or genetic manipulation of mitochondria-shaping proteins, also based on memory T cells.

It needs to be considered that forcing mitochondrial fusion during *in vitro* T cell expansion promotes the differentiation of naive T cells toward a memory phenotype, this conferring

a higher survival to these cells. However, we observed that T cell migration strictly depends on optimal fragmentation of the mitochondrial network; thus, an unbalance toward mitochondria fusion in memory T cells would inhibit their (trans)migratory capability, therefore impinging on their "choice" to be resident or to recirculate. This observation suggests that a one-way or "chronical" modulation of the activity of mitochondria-shaping proteins could hardly result in successful therapeutic strategies, with this highlighting the actual complexity of the topic. Finally, also in a T cell terminal differentiation into senescence, in which cell migration and proliferation are fatally altered, mitochondria structure, and function result impaired as well (47).

TISSUE REGULATORY T CELLS: RESIDENT OR RECIRCULATING?

Most of the available information about resident T cells come from the study of CD8 Trm, and a growing body of data demonstrates their key role in response to pathogens, in antitumor immunity, in mucosal defense, in vaccine efficacy, and so forth [reviewed in (10)]. Less clear are the identity and functions of CD4 Trm in different contexts, probably because in tissues the CD4 T cell population may comprise variable proportions of Tregs displaying completely different immune functions. Tregs represent a class of CD4 T cells defined by the expression of Foxp3 and exerting non-redundant immunosuppressive and tissue repair functions. In several non-lymphoid tissues, Treg subtypes have been identified that show tissue-specific profiles, differentiate locally in response to variable signals, and perform specialized functions [reviewed in (48)].

Whether tissue Tregs are truly resident cells is still a matter of investigation. Parabiosis experiments have demonstrated that Treg chimerism was lower in the adipose tissue and intestine compared to the spleen, blood, and liver (49–51). When Tregs were further classified into central or effector cells, the latter were found more resistant to recirculation (52, 53); however, this event was transient (52), and upon parabiont disconnection, the chimerism of both effector and central Tregs decayed in a few weeks (52). These results suggest that, at least in certain tissues, effector Tregs may be continuously replenished from circulating Tregs, which locally differentiate and proliferate (54).

When effector Tregs were further subdivided according to the expression of the CD49b integrin, it was possible to distinguish circulating Tregs: indeed, compared to other districts, the blood and highly vascularized tissues (liver and lung) contained a high frequency of $CD49b^+$ effector Tregs that displayed a significantly higher rate of exchange between parabiotic mice (55). It could be hypothesized that $CD49b^+$ Tregs may be devoted to continuous tissue patrolling through blood circulation, being able to promptly reach damaged or inflamed tissues (55), while the $CD49b^-$ cells may show a certain degree of stable residency and exert on-site repair/regenerative functions in physiological settings. For instance, Tregs localize to the epithelial stem cell niche and promote hair growth at the steady state (56). Resident Tregs may exist in the heart protecting from fortuitous inflammation and tissue damage (57). Such tiny and highly specialized Treg populations are settled in locations that are poorly accessible to the circulation and, thus, probably may have acquired better capacities to survive and self-renew locally.

Tregs, or certain Treg subsets, share with Trm some phenotypical markers. For instance, Tregs express CD69 at a higher level in non-lymphoid than in lymphoid tissues (58–60). The expression of CD103 by effector Tregs was established several years ago (61), and $CD103^+$ Tregs have been observed at the steady state in several tissues including the lung (58) and the dermis (62). CD39 is a well-recognized marker of Tregs from lymphoid organs (63) and maintained at high levels in tissues like VAT (64). Notably, one of the key transcription factors for the acquisition of a residency program, Blimp1 (65), plays a well-recognized function in the instruction of the effector program in Treg (66). Therefore, in tissues, effector Tregs possess the whole armamentarium that may be needed to establish residency. In this context, a recent paper has shown that the majority of lung-resident CD4 T cells are indeed composed of Tregs that play tissue-protective functions (58).

More elusive is the extent of Treg residency in human tissues. Tregs can be found in several healthy human tissues such as the intestine, skin, adipose tissue, and skeletal muscle (48). In healthy human skin, arginase 2 expression was found as a feature of resident Tregs (67). Whether Tregs can establish long-term residency in these tissues and whether this process may be modified in pathologic conditions remain unclear. Recent analyses in human lung transplant recipients have demonstrated that, contrary to conventional T cells, most Tregs in the bronchoalveolar lavage were of recipient origin (68): this result underscores the dominance of Treg colonization from the blood over persistent Treg residency, at least in this context. According to the mouse data mentioned above (55), it could be suggested that the lung, as a highly vascularized tissue, may be particularly prone to Treg replenishment from the blood and that Treg residency may be more stringent in less vascularized tissues.

The balance between Treg residency and recirculation may have key implications during tissue modifications occurring in chronic inflammation and cancer. Tumor Tregs display a gene signature that combines tissue-specific and tumor-specific genes [reviewed in (69)], and a "core signature" is shared among Tregs infiltrating diverse human cancers (70). In human melanoma, Tregs express a higher level of arginase 2 than in healthy skin (67), suggesting that tumor Tregs may co-opt and enforce signals that preexisted in Tregs resident in the normal parenchyma. In human breast cancer and colon cancer, tumor Tregs were much more similar to the corresponding healthy tissue Tregs than to circulating Tregs (71, 72). However, the analysis of the TCR repertoire of tumor and tissue Tregs led to conflicting results in different tumor types (70–72), and whether tumor Tregs derive from the amplification of Treg clones populating normal tissues, rather than from circulating cells, remains to be ascertained. A deeper understanding of the tumor Treg complexity will be key to designing Treg-targeted therapies that would spare physiological functions of tissue Tregs.

DISCUSSION

T cell heterogeneity comprises not only a great variety of T cell subpopulations with different functions but also a considerable diversity of migratory patterns. These patterns are strongly related to the function that these cells will exert in a specific tissue. After activation, changes in T cell migratory capacity occur simultaneously with cell expansion and differentiation into effectors and memory cells. Noteworthy is the evidence that cycling antigen-specific T cells are present in the blood in the acute phase of the response, suggesting a very dynamic interplay between cell cycle and migration (30–32). Nevertheless, how clonal expansion and migration are related is still unclear. Interestingly, the elderly show an altered T cell clonal expansion and a worse T cell response to infections and vaccination. However, only a few studies have focused on the possible impact of aging on T cell recirculation (73, 74), and a possible relation is still unclear.

Whether T cells recirculate or reside in one tissue strongly depends on their metabolism: indeed, mitochondrial dynamics regulate T cell migration and differentiation (39, 40, 44). Metabolism could also dictate the survival of certain Trm, i.e., resident Tregs, which exert important tissue homeostatic functions (48). However, in some pathological conditions such as tumors, whether infiltrating Tregs derive from the resident population or are mobilized from the circulating pool remains unclear (70, 72). This review highlights novel concepts of T cell compartmentalization and opens new interesting perspectives regarding the regulation of this process both in physiological and in pathological conditions.

AUTHOR CONTRIBUTIONS

SP conceived the review structure. SC prepared the figure. All authors wrote the manuscript.

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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.

APPENDIX: EFIS-EJI Ruggero Ceppellini Advanced School of Immunology Founded by Serafino Zappacosta. List of the Activities From Its Foundation in 1991 to 2019

Note: for ongoing activities see www.ceppellini.it.

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LEVEL 1 COURSES

Level 1 courses are immunology refresher courses aimed at updating Italian physicians, pharmacists, or professionals in other medical disciplines. Notably, the Ruggero Ceppellini Advanced School of Immunology started organizing these courses long before Continuing Medical Education (CME) became mandatory for MDs in Italy in 2002.

L'Immunità in patologia umana 95/96 - Immunity in human pathology 95/96

National Tumor Institute "Giovanni Pascale" of Naples, Naples, November 1995-March 1996

L'Immunità in patologia umana 1999 - Immunity in human pathology 1999

Federico II University Medical School, Naples February - May 1999

L'Immunità in patologia umana 2002 - Immunity in human pathology 2002

Azienda Ospedaliera V Monaldi, Naples, November-December 2002

L'Immunità in patologia umana 2012 - Immunity in human pathology 2012

Federico II University Medical School, Naples, September - December 2012

Immunodeficienze in Pediatria - Immunodeficiency in Pediatrics

Federico II University Medical School, Naples, February - April 2015

LEVEL 2 COURSES

Level 2 courses are the typical activites of the Ceppellini School. They are International Advanced Immunology courses dedicated to young researchers (PhD students, post-docs, etc.) from all over the world, particularly from developing countries, wishing to acquire in-depth knowledge on a specific topic from leader scientists in the field.

"Immunology of Bone Marrow Transplantation"

Palazzo Serra di Cassano, Istituto Italiano per gli Studi Filosofici, Naples,
12-16 October 1992

This Course followed immediately the School's inaugural ceremony at the Istituto Italiano per gli Studi Filosofici. Therefore, the course took place in an atmosphere of emotion and hope and benefited from the direction of Elizabeth Simpson, who played the role of enthusiastic organizer, continuing since then to collaborate lovingly with the School in both the didactic and the organizational aspects.

The Course Director

Elizabeth Simpson
Division of Transplantation Biology, MRC Clinical Research Centre, Harrow, Middlesex, UK

The Faculty

Andrea Bacigalupo
Divisione di Ematologia ed Immunologia Clinica, Ospedale San Martino, Genova, Italy
Giovanni B Ferrara
Servizio di Immunogenetica, Istituto Nazionale Tumori, Genova, Italy
Manlio Ferrarini
Servizio di Immunologia Clinica, Istituto Nazionale Tumori, Genova, Italy
Peter M Hoogerbrugge
Department of Gene Therapy, Institute for Applied Radiobiology and Immunology, Rijswijk, The Netherlands
Jill M Hows
Department of Hæmatology, Southmead Hospital, Bristol, UK
Robert I Lechler
Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK
Guido Lucarelli
Divisione di Ematologia e Centro Trapianto Midollo Osseo di Muraglia,
Ospedale di Pesaro, Pesaro, Italy
Yair Reisner
Department of Biophysics & Membrane Research, The Weizmann Institute of Science, Rehovot, Israel
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DNAX Research Institute, Palo Alto, CA, USA
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Divisione di Ematologia Clinica, Facoltà di Medicina e Chirurgia,
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The Organizing Committee: Antonio Di Giacomo, Azienda Ospedaliera V Monaldi, Napoli, Italy.

Secretariat: Tricia Reynolds, Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy; Vivien Tikerpae, *Division of Transplantation Biology, MRC Clinical Research Centre, Harrow, Middlesex, UK*

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Becton Dickinson Italia SpA, Milano; Boehringer Mannheim Italia SpA, Milano; Heraeus SpA, Cavenago Brianza MI; ICN Biomedical SpA, Cassina de' Pecchi FI; Italfarmaco SpA, Milano; Janssen Farmaceutici SpA, Roma; Lagitre Srl, Milano; M-Medical Srl, Firenze; Microglass Srl, Napoli; Società Prodotti Antibiotici SpA, Milano; Zambon Group SpA, Milano; and the Azienda Autonoma di Soggiorno Cura e Turismo of Naples.

"Progress and Perspectives in Vaccination"

Palazzo Serra di Cassano, Istituto Italiano per gli Studi Filosofici, Naples,
16-20 May 1994

The Course dealt with the most recent aspects of vaccination. Basic knowledge about the immune response as well as new vaccine technology were covered. The Course included an Opening and a Closing Session addressed to the general public on "The Impact of Vaccination on Human Welfare and Society".

The Course Director

Gino Doria
Laboratorio di Immunologia, ENEA-CRE Casaccia, Roma, Italy

The Faculty

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International Centre for Genetic Engineering and Biotechnology, UNIDO, Trieste, Italy
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Afdeling Immunologie, Het Nederlands Kanker Instituut, Amsterdam, The Netherlands
Andrew J McMichael
Institute of Molecular Medicine, John Radcliffe Hospital, Oxford University, Oxford, UK
Filippo Palumbo
Osservatorio Epidemiologico Regionale, Regione Campania, Napoli, Italy
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Divisione di Oncologia Sperimentale D, Istituto Nazionale Tumori, Milano, Italy
Marcello Piazza
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The Organizing Committee: Antonio Di Giacomo, *Azienda Ospedaliera V Monaldi, Napoli, Italy;* Ciro Manzo & Armando Tripodi, *Istituto Nazionale Tumori Fondazione Pascale, Napoli, Italy;* Luigi Racioppi & Serafino Zappacosta, *Università di Napoli Federico II, Napoli, Italy.*

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Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Istituto Nazionale Tumori Fondazione Pascale Naples, Italy; Biocine Srl, Siena; Bio-Rad SpA, Milano, and Microg

"T-Cell Activation, Anergy and Immunosuppressive Drug Action"

Istituto Nazionale Tumori Fondazione Pascale, Naples,
17-21 October 1994

This Course covered the main aspects of T cell activation, central to all mechanisms of the immune response, and it included an Opening session on "The continuing education of physicians and researchers", addressed to a local audience.

The Course Director

Stefan C Meuer
Abteilung Angewandte Immunologie, Deutsches Krebsforschungszentrum Heidelberg, Heidelberg, Germany

The Faculty

V Enrico Avvedimento
Dipartimento di Medicina Sperimentale e Clinica, Università di Reggio Calabria, Catanzaro, Italy
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Mario Condorelli
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Bernhard Fleischer
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The Organizing Committee: Antonio Di Giacomo, *Azienda Ospedaliera V Monaldi, Napoli, Italy;* Ciro Manzo & Armando Tripodi, *Istituto Nazionale Tumori Fondazione Pascale, Napoli, Italy;* Serafino Zappacosta, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Tricia Reynolds (Chairperson) & Carla Corradini, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy;* Margherita Foggia & Francesco Scerbo, I*stituto Nazionale Tumori Fondazione Pascale, Napoli, Italy;* Amanda Wren, *Nature Classified, Macmillan Magazines Ltd, London, UK.*

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Istituto Nazionale Tumori Fondazione Pascale Naples, Italy; Dianova GmbH, Hamburg, Germany, and from Hermann Biermann GmbH, Bad Neuheim, Germany.

"Immunity to Intracellular Bacteria & Parasites"

Positano, Hotel Le Agavi, near Salerno,
20-25 May 1995

The objective of this Course was to spread information on infectious disease pathogenesis and immunity. About 35% of the world population still die of infections. Thus, there is a great demand for improvement. A prerequisite is a sound understanding of basic mechanisms of pathogenesis and immunity to infections. Leading scientists in these fields contributed to make this course a success.

The Course Director

Helmut Hahn
Institut für Medizinische Mikrobiologie und Infektionsimmunologie,
Freie Universität Berlin, Berlin, Germany

The Faculty

Peter Andersen
Bacterial Vaccines Department, Statens Seruminstitut, Copenhagen, Denmark
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The Organizing Committee: Antonio Di Giacomo, *Azienda Ospedaliera V Monaldi, Napoli, Italy;* Stefan Ehlers & Martin E A Mielke, *Freie Universität Berlin, Berlin, Germany;* Luigi Racioppi & Serafino Zappacosta, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Tricia Reynolds, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy.*

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Dipartimento di Biologia e Patologia Cellulare e Molecolare of the Federico II University of Naples; Bio-Rad Srl, Segrate MI; Microglass Srl, Napoli; Bayer (Germany); Lederle Pharma (Wolfratshausen, Germany); and Takeda Pharma (Aachen, Germany); Assessorato al Turismo e Spettacolo of the Campania Region.

"Mechanisms and Manipulation of Autoimmunity"

Istituto Nazionale Tumori Fondazione Pascale, Naples,
23-27 June 1996

This Course was devoted to the presentation and discussion of the essential aspects of autoimmunity, of remarkable interest from both the speculative and the clinical standpoints. After a review of the immunological tolerance and of the antigen presentation mechanisms, the course focussed on several clinical aspects, from the genetic susceptibility to recent therapeutic approaches to autoimmune diseases.

The Course Director

Robert I Lechler
Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK

The Faculty

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Department of Pathology, Immunology Division, University of Cambridge, Cambridge, UK
Rikard Holmdahl
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Department of Anatomy, Medical School, University of Birmingham, Birmingham, UK
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The Organizing Committee: Silvia Fontana, *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Ciro Manzo & Armando Tripodi, *Istituto Nazionale Tumori Fondazione Pascale, Napoli, Italy;* Guido Rossi, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Margherita Foggia, Tiziana Foggia & Francesco Scerbo, *Istituto Nazionale Tumori Fondazione Pascale, Napoli, Italy;* Amanda Wren, *Nature Classified, Macmillan Magazines Ltd, London, UK.*

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Istituto Nazionale Tumori Fondazione Pascale, Naples, Italy; Amersham Italia Srl, Milano; Dasit SpA, Cornaredo MI; International PBI SpA, Milano; Microglass Srl, Napoli; and M-Medical Srl, Firenze.

"HLA & Tumours"

Istituto Nazionale Tumori Fondazione Pascale, Naples,
2-6 December 1996

This Course was aimed at the presentation and discussion of some immunological aspects of tumor-host interaction, including the control exerted by the innate and adaptive immune responses. Therefore, the attention was focussed on the potential therapeutic approaches based on exploiting anti-tumour immunity.

The Course Directors

Soldano Ferrone
Department of Microbiology & Immunology, New York Medical College, Valhalla, NY, USA
Ciro Manzo
Divisione di Oncologia Sperimentale C, Immunologia, Istituto Nazionale Tumori Fondazione Pascale, Napoli, Italy

The Faculty

Ennio Carbone
Cattedra di Immunologia, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II, Napoli, Italy
Marco Colonna
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John Guardiola
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Rolf Kiessling
Microbiology & Tumorbiology Center, Karolinska Institutet, Stockholm, Sweden
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Francesco M. Marincola
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Lorenzo Moretta
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Barbara Seliger
III Medizinische Klinik, Abteilung für Innere Medizin-Hämatologie,
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Human Immunogenetics Laboratory, Imperial Cancer Research Fund, London, UK

The Organizing Committee: Antonio Di Giacomo, *Azienda Opedaliera V Monaldi, Napoli, Italy;* Silvia Fontana, *Centro di Endocrinologia e Oncologia, Sperimentale, CNR, Napoli, Italy;* Giuseppina Ruggiero, *Università di Napoli Federico II, Napoli, Italy;* Armando Tripodi, *Istituto Nazionale Tumori Fondazione Pascale, Napoli, Italy.*

Secretariat: Maria de Manes & Alessandra Saioni, Effe Erre Congressi, Napoli, Italy; Anna Maria Masci & José Terrazzano, Università di Napoli Federico II, Napoli, Italy.

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Istituto Nazionale Tumori Fondazione Pascale, Naples, Italy; Dako SpA, Milano; Labscience Italia Srl, Torre del Greco NA; and Microglass Srl, Napoli.

Cytokines in Immunity

Città della Scienza, Naples,
3-7 November 1997

This Course dealt with the structural and functional properties of cytokines and their receptors in the complex regulatory circuits of immunity. The timeliness of such Course in a period characterised by an explosive outbreak of information both on the physiological and on the pathological implications of the immune response was witnessed by the enthusiastic participation of a very distinguished faculty and of a large international audience.

The Course Director

Abul K Abbas
Department of Pathology, Immunology Research Division, Brigham and Women's Hospital, Boston, MA, USA

The Faculty

Abul K Abbas
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Sponsorships: IDIS Foundation-Città della Scienza; Istituto Italiano per gli Studi Filosofici, Naples, Italy; Istituto Nazionale Tumori Fondazione Pascale, Naples, Italy; the Campania Region (Research Service); the Federico II University of Naples; Amersham Italia Srl, Milano; Becton Dickinson Italia SpA, Milano; Corning Costar Italia Srl, Conrorezzo MI; DBA Italia Srl, Segrate MI; Dia-Chem Srl, Napoli; EG&G SpA, Milano; Eppendorf Srl, Milano; Immucor Italia Srl, Noversasco di Opera MI; Internationl PBI SpA, Milano; Microglass Srl, Napoli; Perkin Elmer Italia SpA, Monza MI; Primm Srl, Milano; Schering-Plough SpA, Milano; Sigma Aldrich Srl, Milano; Tema Ricerca Srl, Bologna.

"Dendritic Cell Physiology"

Hotel Le Agavi, Positano, near Salerno,
20-24 May 1999

This Course was dedicated to one of the the most central topics of immunology, the interest in which was enhanced by the potential applications of dendritic cells in all interventions on the immune system. The Course benefited highly from the enthusiastic role played by Paola Ricciardi Castagnoli, who was able to gather the best faculty available, running then an impressive sequence of lectures. A Round Table on "In vivo Veritas: Integration of in vitro and in vivo Models of Dendritic Cell Physiology" was organised by Ian McConnell & Elizabeth Simpson and run in the afternoon of the last day of the Course.

The Course Director

Paola Ricciardi Castagnoli
Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Milano, Italy

The Faculty

Sebastian Amigorena
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Francine Brière
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The Organizing Committee: Silvia Fontana, *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Giuseppina Ruggiero, José Terrazzano & Serafino Zappacosta, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Maria de Manes, Alessandra Saioni & Daniela Giampaolo, *Effe Erre Congressi, Napoli, Italy;* Nevin Bayoumi, *Nature Classified, Macmillan Magazines Ltd, London, UK.*

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Università di Napoli Federico II; the Campania Region Education Service, the Mayor of Positano; Immunex Corporation, Seattle, WA, USA; Miltenyi Biotech GmbH, Bergisch Gladbach, Germany; Roche SpA, Milano; Tema Ricerca Srl, Bologna, and The Ares Serono Group, Geneva, Switzerland.

"Escape From Immune Surveillance of Tumours and Micro-organisms: Emerging Mechanisms and Shared Strategies"

Centro S. Ignazio, Naples,
23-27 March 2000

The Course was aimed at discussing in a number of systems the molecular mechanisms behind the immunological escape of tumours, viruses, bacteria and parasites, accounting for their ability to evade detection by T cells, NK cells and antibodies. In particular, selection of antigenic loss variants, defects in antigen presentation, immune suppressive cytokines, defects in signal transducing molecules, induction of apoptosis in T cells and loss of cytokine receptors were discussed.

The Course Directors

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The Organizing Committee: Silvia Fontana, *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Giuseppina Ruggiero, José Terrazzano & Serafino Zappacosta, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Nevin Bayoumi, *Nature Classified, Macmillan Magazines Ltd, London, UK;* Valeria Lamorgese, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy;* Alessandra Saioni & Daniela Giampaolo, *Effe Erre Congressi, Napoli, Italy.*

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Università di Napoli Federico II; Swedish Cancer Society of Stockholm; Becton Dickinson Italia SpA, Milano; Microglass Scientific Apparatus snc, Napoli; PBI International SpA, Milano; Valter Occhiena Srl, Torino.

"Remembering Environmental Experiences: The Physiological Basis of Memory in the Immune and Nervous Systems"

Cala Moresca Hotel Club, Capo Miseno, near Naples,
28 June 28- 2 July 2001

The course reviewed the mechanisms generating and maintaining memory at the cellular and biochemical levels in both the immune and nervous systems, to show similarities and differences between these two interfaces of the host with the external environment. Issues such as the need for repetitive stimulation to maintain memory, the role played by changes in cellular differentiation in providing effective memory, and the biochemistry of the memory state were reviewed both in the immunology and the neurobiology fields, looking for common themes in the biology of learning.

This activity of the Ceppellini School was dedicated to the memory of Alfred Nisonoff, one of the most relevant immunologists of past Century and one of the founders of the School, suddenly deceased on March 12, 2001.

The Course Directors

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The Organizing Committee: Silvia Fontana, *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Silvestro Formisano, Veronica Sanna & José Terrazzano, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Nevin Bayoumi, *NatureJobs, Macmillan Magazines Ltd, London, UK;* Pina Ippolito, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy;* Daniela Giampaolo & Ornella Spada, *Effe Erre Congressi, Napoli, Italy;* Sarah Green, *British Society for Immunology, London, UK.*

Sponsorships: Istituto Italiano per gli Studi Filosofici, Naples, Italy; Università di Napoli Federico II; the Campania Region Education Service; Microglass Scientific Apparatus snc, Napoli.

"Physiology of the Mucosal Immune Response"

Cala Moresca Hotel Club, Capo Miseno, near Naples,
18-22 October 2001

The Course discussed the most recent advances in the mucosal immune response, particularly the intestinal immune response. Emphasis was given to the physiological aspects of the mucosal immune system, but the course covered also vaccine development and some of the diseases characterised by dysregulated mucosal immunity.

Like the preceding course held in the same year, the Course was dedicated to the memory of Alfred Nisonoff.

The Course Directors

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The Organizing Committee: Salvatore Auricchio, Silvestro Formisano, & Riccardo Troncone, *Università di Napoli Federico II, Napoli, Italy;* Francesca Di Rosa, *Istituto Internazionale di Genetica e Biofisica, CNR, Napoli, Italy.*

Secretariat; Nevin Bayoumi, *NatureJobs, Macmillan Magazines Ltd, London, UK;* Pina Ippolito, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy;* Daniela Giampaolo & Ornella Spada, *Effe Erre Congressi, Napoli, Italy;* Sarah Green, *British Society for Immunology, London, UK.*

Sponsorhsips: European Laboratory for the Investigation of Food-Induced Diseases of the Università di Napoli Federico II; Istituto Italiano per gli Studi Filosofici, Naples, Italy; Research Service of the Campania Region.

"The Immune System in the Protection and Susceptibility to Tuberculosis"

Cala Moresca Hotel Club, Capo Miseno, near Naples, and Naples,
13-16 September 2002

The Course reviewed the most recent advances in the immunological aspects of tuberculosis research and treatment. Emphasis was given to the role of innate and acquired immunity to the pathogen, to the molecular biology of mycobacteria, as well as to novel vaccination strategies.

The 10th Anniversary of the Ceppellini School of Immunology was celebrated with a Special Session on Sept 13 in Naples.

The Course Directors

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The Organizing Committee: Francesca Di Rosa, *Istituto Internazionale di Genetica e Biofisica A Buzzati-Traverso, CNR, Napoli;* Silvestro Formisano & Giuseppina Ruggiero, *Università di Napoli Federico II, Napoli;* Pina Ippolito, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli.*

Secretariat: Daniela Giampaolo & Roberta De Marco, *Effe Erre Congressi, Napoli;* Silvana Aprile, *Istituto Italiano per gli Studi Filosofici, Napoli;* Stacey Sheekey, *Sales Support Manager, Current Trends, Elsevier Science, London, UK.*

Sponsorships: Assessorato alla Ricerca of the Campania Region; Federico II University of Naples; Istituto Italiano per gli Studi Filosofici, Naples; UNESCO; Lazzaro Spallanzani Institute, Rome.

"Mucosal Immunity 2: Mucosal Infection and Inflammation"

Cala Moresca Hotel Club, Capo Miseno, near Naples,
10-14 October 2002

This Course was intended to move forward the first Ruggero Ceppellini Course on Basic Mechanisms of Mucosal Immunity held in 2001, by exploring the mechanisms involved in the local immune defence of mucosal surfaces against infection, as well as those responsible for chronic inflammatory conditions of the intestine. The Course focussed on the interactions between epithelial cells and pathogenic/commensal organisms and the resulting effects on local inflammatory cells. The pathogenic mechanisms of chronic inflammatory bowel disease, as well as of coeliac disease, were discussed.

The Course Director

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The Faculty

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The Organizing Committee: Francesca Di Rosa, *Istituto Internazionale di Genetica e Biofisica A Buzzati-Traverso, CNR, Napoli;* Silvestro Formisano, Salvatore Auricchio & Riccardo Troncone, *Università di Napoli Federico II, Napoli,* Pina Ippolito, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli.*

Secretariat: Daniela Giampaolo and Associates, *Effe Erre Congressi, Napoli;* Stacey *Sheekey, Sales Support Manager, Current Trends, Elsevier Science, London, UK;* Doreen Shand; *British Society for Immunology, London, UK.*

Sponsorships: European Laboratory for the Investigation of Food Induced Diseases of the Federico II University of Naples; Assessorato alla Ricerca of the Campania Region; Istituto Italiano per gli Studi Filosofici, Naples; Società Italiana di Storia Patria di Terra di Lavoro; Microtech srl, Naples.

"The Stem Cell: From Theory to Clinics"

Lloyd's Baia Hotel, Vietri sul Mare, near Salerno,
16-20 October 2003

The recent discovery of stem cells in many human tissues has raised high hope for the cure of many genetic and acquired diseases. This course addressed the basic concepts of stem cells, including topics such as human embryonic stem cell lines, stem cell plasticity, molecular control of stem cell proliferation, differentiation and plasticity, stem cell manipulation in vitro. The Course included a round table discussion on "Ethycal issues in human embryonic cell research".

The Course Directors

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Secretariat: Pina Ippolito & Riccardo Zappacosta, *Ceppellini School Secretarial Office.*

Sponsorships: CEINGE Biotecnologie Avanzate Scarl, Naples; Federico II University of Naples; Assessorato alla Ricerca of the Campania Region; Istituto Nazionale Tumori Fondazione Pascale, Naples; Istituto Italiano per gli Studi Filosofici, Naples; Microtech srl, Naples, from Becton Dickinson SpA, Buccinasco (Milan) and from Instrumentation Laboratory, Rome.

"Innate Immunity in Self and Infectious Non-Self Recognition"

Cala Moresca Hotel Club, Capo Miseno, near Naples,
10-14 March 2005

The course reviewed the most advanced knowledge about innate immune mechanisms at the genetic, cellular and molecular levels. Addressed topics included the innate immune cells and receptors in viral, bacterial, fungal and parasitic infections; the genomic analysis of innate immunity receptor families, the pathogen recognition by insect vectors of human infectious diseases, the molecules recognising pathogen Toll and non-Toll-like, the signalling events leading to NK cell activation, the NK cell-associated receptors and their role in immune regulation, the interactions between innate and adaptive immunity, the possibility of recognizing cancer with innate receptors, and new therapeutic approaches to infectious diseases.

The Course Directors

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Secretariat: Alessandra Saioni, Mia Liotti and Associates, *Effe Erre Congressi, Napoli, Italy;* Sille Opstrup, *NatureJobs, Macmillan Magazines Ltd, London, UK.*

Sponsorships: Magna Græcia University of Catanzaro; Federico II University of Naples; Microtech srl, Naples, Instrumentation Laboratory SpA, Rome, Italy, and Becton-Dickinson SpA, Buccinasco Milan.

"The recrudescence of an old infectious disease: Tuberculosis"

Congress Center University of Naples "Federico II", Naples
2-5 May 2007

The course reviewed an infectious disease of increasing global concern, namely tuberculosis. In recent years, strains of Mycobacterium tuberculosis have developed resistance to classical drug therapies which limit its pathological burden. The course reviewed this recent health care emergency with a focus on the genetics of Mycobacterium, the emergence of new drug-resistant strains, and the proteomics of the M. tuberculosis cell wall. The immune response to M. tuberculosis was discussed in-depth by reviewing state-of-the-art research on mechanisms involved in immune control of M. tuberculosis infection. In addition, novel anti-M. tuberculosis vaccines and drug development strategies were discussed.

The Course Director

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The Faculty

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The Organizing Committee: Antonio Di Giacomo, *Azienda Ospedaliera V Monaldi, Napoli, Italy;* Ennio Carbone, *Magna Grecia University, Catanzaro;* Enrico Avvedimento, Guido Rossi & Silvestro Formisano, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Effe Erre Congressi, *Napoli, Italy.*

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II; Magna Grecia University, Catanzaro; Istituto Italiano per gli Studi Filosofici; International Doctorate Program in Molecular Oncology and Endocrinology; Research Service of the Campania Region.

"Tumour Immune Escape 2008"

Circolo dei Forestieri, Sorrento near Naples,
16-18 October, 2008

The Course brought together scientists with various points of view on anti-cancer immune surveillance. During the course, several observations on the role of immunity in controlling tumor progression were reviewed and discussed. Mechanisms involved in immune subversion by established tumors were discussed, as well as novel anti-cancer vaccines and drug development strategies.

The Course Directors

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The Organizing Committee: Antonio Di Giacomo, *Azienda Ospedaliera V Monaldi, Napoli, Italy;* Ennio Carbone, *Magna Grecia University, Catanzaro;* Enrico Avvedimento, Guido Rossi & Silvestro Formisano, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Effe Erre Congressi, *Napoli, Italy.*

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II; Magna Grecia University, Catanzaro; Istituto Italiano per gli Studi Filosofici; International Doctorate Program in Molecular Oncology and Endocrinology; Research Service of the Campania Region.

EFIS-EJI course on "The role of B Cells in the Physiology and Pathology of the Immune System"

Circolo dei Forestieri, Sorrento near Naples,
5-7 November 2009

This Course dealth with recent advancements in the B cell field. Topics included B cell development, B cell diversification and memory formation, B cell involvement in autoimmune diseases.

The Course Directors

Antonio La Cava
Lupus Research Laboratory, University of California Los Angeles, California, USA
Andreas Radbruch
German Rheumatology Research Center, Leibniz Research Institute, Berlin, Germany

The Faculty

Marina Botto
Rheumatology Section, Faculty of Medicine, Imperial College, London, UK
Rita Carsetti
Ospedale Pediatrico Bambino Gesù, Rome, Italy
Falk Hiepe
Charitè University of Medicine, Berlin, Germany
Antonio La Cava
Lupus Research Laboratory, University of California Los Angeles, California, USA
Ian MacLennan
MRC Centre for Immune Regulation, Univerity of Birmingham, Birmingham, UK
Fritz Melchers
Max Planck Institute for Infection Biology, Berlin, Germany
Andreas Radbruch
German Rheumatology Research Center, Leibniz Research Institute, Berlin, Germany
Antonius Rolink
Developmental and Molecular Immunology, University of Basel, Switzerland
Elisabetta Traggiai
Institute G. Gaslini, Genova, Italy
Jean-Claud Weill
Paris Descartes University, Site Necker-Enfants Malade, Paris, France

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Ennio Carbone, *Magna Grecia University, Catanzaro;* Guido Rossi & Silvestro Formisano, *Università di Napoli Federico II, Napoli, Italy;* Antonio La Cava, *Lupus Research Laboratory, University of California Los Angeles, California, USA;* Tricia Reynolds, *Intercultural Relations Center, Naples, Italy.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); the Federico II University of Naples; the Magna Graecia University of Catanzaro; the Research Service of Campania Region; the Istituto Italiano per gli Studi Filosofici, Naples, Italy; the Department of Cellular and Molecular Biology and Pathology, Federico II University of Naples; the International Doctorate Program in Molecular Oncology and Endocrinology of Naples; the International Doctorate Program in Molecular Oncology, Immunology and Development of New Therapy of Catanzaro, Italy.

EFIS-EJI course on "Innovative stategies for vaccines: malaria, tuberculosis, HIV"

Circolo dei Forestieri, Sorrento near Naples,
4-6 November 2010

The Course highlighted the current views and state of the art of vaccine production against three dangerous infectious diseases, i.e. malaria, tuberculosis, HIV/AIDS, that taken together are responsible for millions of deaths globally. Future developments in the area were discussed.

The Course Directors

Stefan H.E. Kaufmann
Max Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany
Rino Rappuoli
Novartis Vaccines, Siena, Italy
Giuseppe del Giudice
Novartis Vaccines, Siena, Italy

The Faculty

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Clinical Research & Translational Science, GSK Vaccine, Rixensart, Belgium
Giuseppe del Giudice
Novartis Vaccines, Siena, Italy
Peter Andersen
Department of Infectious Disease Immunology, Statens Serum Institute, Copenhagen, Denmark
Susan W Barnett
Novartis Vaccines and Diagnostics, Cambridge, MA, USA
Bertram L Jacobs
Arizona State University, Tempe, Arizona, USA
Stefan H.E. Kaufmann
Max Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany
Kai Matuschewski
Max Planck Institute for Infection Biology, Parasitology Unit, Berlin, Germany
Martin OC Ota
Bacterial Diseases Programme, MRC Laboratories, Banjul, Gambia
Rino Rappuoli
Novartis Vaccines, Siena, Italy
Eleanor M Riley
Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK
Alexander von Gabain
Intercell AG, Vienna, Austria
Barry Walker
National Institute for Biological Standards and Controls, Potters Bar, Hertfordshire, UK.
Hedda Wardemann
Molecular Immunology Research Group, Max Planck Institute for Infection Biology, Berlin, Germany
Fidel Zavala
Johns Hopkins Malaria Research Institute and Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Baltimore, Maryland, USA

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Antonio Di Giacomo (*Azienda Ospedaliera V Monaldi, Napoli, Italy*); Ennio Carbone, *Magna Grecia University, Catanzaro, Italy;* Guido Rossi & Silvestro Formisano, *Università di Napoli Federico II, Napoli, Italy;* Tricia Reynolds, *Intercultural Relations Center, Naples, Italy.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II; Magna Grecia University, Catanzaro; Novartis, Siena, Italy; Intercell AG, Austria; GlaxoSmithkline, Belgium.

EFIS-EJI course on "Innovative strategies to prevent transplant rejection"

Circolo dei Forestieri, Sorrento near Naples,
26-29 October, 2011

The Course reviewed and discussed the current views on basic concepts of Immunology of transplantation, as well as clinical perspectives in transplantation and new mechanisms of tolerance induction.

The Course Directors

Robert Lechler
Medical Research Council Centre for Transplantation, King's College London, London, UK
Giovanna Lombardi
Medical Research Council Centre for Transplantation, King's College London, London, UK
Randolph J Noelle
Medical Research Council Centre for Transplantation, King's College London, London, UK

The Faculty

Stephen Cobbold
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK
Anthony Dorling
Medical Research Council Centre for Transplantation, King's College London, London, UK
Maria P. Hernandez-Fuentes
Medical Research Council Centre for Transplantation, King's College London, London, UK
Rachel Hilton
Medical Research Council Centre for Transplantation, King's College London, London, and NIHR Biomedical Research Centre at Guy's and St Thomas' Hospital and King's College London, London, UK.
Claudia Kemper
Division of Immunology, Infection and Inflammatory Diseases, King's College London, MedicalResearch Council Centre for Transplantation, Guy's Hospital, London, UK
Robert Lechler
Medical Research Council Centre for Transplantation, King's College London, London, UK
Giovanna Lombardi
Medical Research Council Centre for Transplantation, King's College London, London, UK
Randolph J Noelle
Medical Research Council Centre for Transplantation, King's College London, London, UK
Alberto Sanchez-Fueyo
Liver Transplant Unit, Hospital Clinic Barcelona, IDIBAPS, CIBEREHD, University of Barcelona, Barcelona, Spain.
Elizabeth Simpson
Division of Immunology and Inflammation, Imperial College, London, UK
Richard D Smith
Department of Public Health and Policy, London School of Hygiene and Tropical Medicine, London, UK
Terry B Strom
The Transplant Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
Laurence A Turka
The Transplant Institute, Beth Israel-Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.
Kathryn Wood
Nuffield Department of Surgery, University of Oxford, Oxford, UK

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Antonio Di Giacomo, *Azienda Ospedaliera V Monaldi, Napoli, Italy;* Ennio Carbone, *Magna Grecia University, Catanzaro, Italy;* Silvestro Formisano, *Università di Napoli Federico II, Napoli, Italy;* Tricia Reynolds, *Intercultural Relations Center, Naples, Italy.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II; Magna Grecia University, Catanzaro; Miltenyi, UK; Miltenyi, Germany; Novartis, UK.

EFIS-EJI course on "Innate Immunity 2012: from evolution to revolution"

Circolo dei Forestieri, Sorrento near Naples,
31 October-4 November 2012

Innate immunity is essential for anti-pathogen protection, but it also contributes to the pathogenesis of many diseases. The Course reviewed and discussed the enormous body of work that recently revolutionized the field, including for example the discovery of innate receptors, the increasing knowledge on macrophage heterogeneity, etc.

The Course Directors

Klas Kärre
Karolinska Institutet, Stokholm, Sweden
Lorenzo Moretta
Istituto Giannina Gaslini, Genova, Italy
Ennio Carbone
Magna Grecia University, Catanzaro, Italy

The Faculty

Niels Borregaard
The Granulocyte Research Laboratory, Department of Hematology, National University Hospital, University of Copenhagen, Denmark
Ennio Carbone
Magna Grecia University, Catanzaro, Italy
Susanna Cardell
Dept of Microbiology and Immunology, Institute of Biomedicin, University of Goteborg, Sweden
Jonathan Ewbank
Centre d'Immunologie de Marseille-Luminy, Marseille, France
Siamon Gordon
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK
Francesca Granucci
Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy
Dieter Kabelitz
Institute of Immunology, University of Kiel, Germany
Klas Kärre
Karolinska Institutet, Stokholm, Sweden
Ed Lavelle
Adjuvant Research Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College, Dublin, Ireland
Alberto Mantovani
Istituto Clinico Humanitas, University of Milan, Italy
Lorenzo Moretta
Istituto Giannina Gaslini, Genova, Italy
Christian Münz
Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Switzerland
Jean-Marc Reichhart
UPR 9022 CNRS, Strasbourg, France
Francesco Tedesco
Department of Life Sciences, University of Trieste, Italy
Andrea Velardi
Bone Marrow Transplantation Programme, University of Perugia, Italy

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Antonio Di Giacomo, *V Monaldi Hospital, Napoli, Italy;* Ennio Carbone, *Magna Grecia University, Catanzaro;* Tricia Reynolds, *Intercultural Relations Center, Naples, Italy.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Gender Equality and Career Development Committee, International Union of Immunological Societies (IUIS); The Magna Grecia University of Catanzaro, Italy; the Department of Cellular and Molecular Biology and Pathology, Federico II University of Naples; the International Doctorate Program in Molecular Oncology, Immunology and Development of New Therapy of Catanzaro, Italy.

EFIS-EJI Course on "Novel Vaccination Strategies Against the Three Major Killers: the Latest News on Malaria, Tuberculosis, HIV/AIDS and Vaccine Development in general"

Restoring Ancient Stabiae–Vesuvian Institute, Castellammare di Stabia, near Naples,
16 - 20 Oct 2013

This Course was intended to move forward the 2010 Ruggero Ceppellini Course on "Innovative stategies for vaccines: malaria, tuberculosis, HIV", by dissecting host-pathogen interactions, discussing the recent improvements on vaccine design, and exploring new methods for identification of biomarkers of protective responses, among other topics.

The Course Directors

Stefan HE Kaufmann
Max Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany
Francesca Chiodi
Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

The Faculty

W. Ripley Ballou
Clinical Research & Translational Science, GSK Vaccine, Rixensart, Belgium
Francesca Chiodi
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Mark Cotton
Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa
Willem Hanekom
University of Cape Town, South Africa
Ali Harandi
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Stefan HE Kaufmann
Max Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany
Elena A Levashina
Max Planck Institute for Infection Biology, Department of Vector Biology, Berlin, Germany
Kai Matuschewski
Max Planck Institute for Infection Biology, Parasitology Unit, Berlin, Germany
Ndung'u Thumbi
HIV Pathogenesis Programme, Doris Duke Medical Research Institute, and the KwaZulu-Natal Research Institute for Tubercolosis and HIV (K-RITH), University of KwaZulu-Natal, Durban, South Africa
Anne O'Garra
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Rino Rappuoli
Novartis Vaccines and Diagnostics, Siena, Italy
Federica Sallusto
Institute for Research in Biomedicine, University of Lugano (USI), Bellinzona, Switzerland
Marita Troye-Blomberg
The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden
January Weiner
Max Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany
Robin A Weiss
Division of Infection & Immunity, University College London, London, UK
Chris Wilson
Director Discovery & Translational Science, Bill and Melinda Gates Foundation, Seattle, WA, USA

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Antonio Di Giacomo, *V Monaldi Hospital, Napoli, Italy;* Tricia Reynolds, *Intercultural Relations Center, Naples, Italy.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Educational Committee, International Union of Immunological Societies (IUIS); The Bill & Melinda Gates Foundation; the Department of Cellular and Molecular Biology and Pathology, Federico II University of Naples; The Journal of Internal Medicine; Novartis vaccines and Diagnostics; Mabtech.

EFIS-EJI course on "The Maternal Immune System in Pregnancy"

Restoring Ancient Stabiae–Vesuvian Institute, Castellammare di Stabia, near Naples,
6-9 Dec 2014

This course reviewed current views on immunological mechanims allowing pregnant mothers to tolerate their fetus and at the same time to display anti-pathogen protection. Topics included the influence of variation of immune system genes (e.g. HLA, KIR) on human reproduction, and the role of fetal immune system, among others.

The Course Directors

Francesco Colucci
Department of Obstetrics and Ginæcology, University of Cambridge, UK
Ashley Moffett (Cambridge)
Department of Pathology, University of Cambridge, UK

The Faculty

Maria-Luisa Alegre
Section of Rheumatology, Department of Medicine, University of Chicago, IL, USA
Ennio Carbone
Università Magna Græcia, Catanzaro, Italy and Karolinska Institutet, Stockholm, Sweden
Francesco Colucci
Department of Obstetrics and Ginæcology, University of Cambridge, UK
Anthony W De Tomaso
Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA, USA
Alison Elliot
MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda and London School of Hygiene and Tropical Medicine, London, UK
Thomas F Gajewski
Department of Pathology, University of Chicago Medical Center, IL, USA
Jacob Michaëlsson
Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden
Ashley Moffett (Cambridge)
Department of Pathology, University of Cambridge, UK
Annettee Nakimuli
Department of Obstetrics and Ginæcology, Makerere University, Kampala, Uganda
Angela Santoni
Department of Molecular Medicine, Sapienza University, Rome, Italy
Elizabeth Simpson
Division of Immunology and Inflammation, Imperial College, London, UK
Tamara Tilburgs
Department of Stem Cells and Regenerative Biology, Harvard University, Cambridge, MA, USA
John Trowsdale
Division of Immunology, Department of Pathology, University of Cambridge, UK

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Centro di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Antonio Di Giacomo, *V Monaldi Hospital, Napoli, Italy;* Tricia Reynolds, *Scientific Secretariat, Scuola Superiore d'Immunologia Ruggero Ceppellini.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); the Educational Committee and the Gender Equality and Career Development Committee, International Union of Immunological Societies (IUIS); The Bill & Melinda Gates Foundation; the Department of Cellular and Molecular Biology and Pathology, Federico II University of Naples.

EFIS-EJI Course on "Treg Biology and Metabolism"

Grand Hotel Oriente, Naples,
5-6 November 2015

Regulatory T cell (Treg) biology was reviewed, particularly in the context of anti-transplant immunity. Treg metabolic programs, and their influence on Treg function were some of the topics discussed in this Course.

The Course Directors

Fiona Powrie
Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK
Giovanna Lombardi
MRC Centre for Transplantation, King's College London, London, UK
Giuseppe Matarese
Laboratorio di Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy

The Faculty

Hogbo Chi
Department of Immunology, St Jude Children's Research Hospital, Memphis, TN, USA
Marika Falcone
IRCSS San Raffaele Hospital, Milan, Italy
Giovanna Lombardi
MRC Centre for Transplantation, King's College London, London, UK
Graham Lord
MRC Centre for Transplantation, King's College London, London, UK
Federica Marelli-Berg
William Harvey Research Institute, Barts, and London School of Medicine and Dentistry, London, UK
Giuseppe Danilo Dorata
Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy
Erika L. Pearce
Department of Immunometabolism, Max Planck Institute for Immunobiology and Epigenetics, Freiburg, Germany
Fiona Powrie
Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Istituto di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Antonio Di Giacomo, *V Monaldi Hospital, Napoli, Italy;* Tricia Reynolds, *Scientific Secretariat, Scuola Superiore d'Immunologia Ruggero Ceppellini.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); the Gender Equality and Career Development Committee, International Union of Immunological Societies (IUIS); the Department of Translational Medicine, Federico II University of Naples; European Research Council (ERC); .M&M Biotech; Space Import & Export srl; Seahorse Bioscience; Euroclone SpA.

EFIS-EJI Course on "Metchnikoff's Legacy: tissue Phagocytes and Functions"

Stazione Zoologica "Anton Dohrn", Naples,
12-14 October 2016

This Course brought together scientists with various perspectives on macrophages, their heterogeneity, and their role in immune responses, e.g. against M. tuberculosis. The pioneer studies of Metchnikoff, who first described phagocytosis, were mentioned and discussed in the light of current literature in the field.

The Course Directors

Siamon Gordon
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK
Stefan H E Kaufmann
Max-Planck-Institut für Infektionsbiologie, Berlin, Germany
Fernando Martinez Estrada
University of Surrey, Guildford, Surrey, UK

The Faculty

Vincenzo Bronte
Sezione di Immunologia Department of Medicine Verona University Hospital Verona, Italy
Cecilia Garlanda
Laboratory of Experimental Immunopathology, Istituto Clinico Humanitas – IRCCS, Rozzano, Milan, Italy
Diego Gomez-Nicola
Biological Sciences, University of Southampton Southampton General Hospital, Southampton, UK
Siamon Gordon
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK
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Wellcome Trust, Institute of Cellular Medicine Newcastle University, Newcastle upon Tyne, UK
Branka Horvat
International Center for Infectiology University of Lyon, Lyon, France
Stefan H E Kaufmann
Max-Planck-Institut für Infektionsbiologie, Berlin, Germany
Elzbieta Kolaczkowska
Department of Evolutionary Immunobiology, Institute of Zoology Jagiellonian University, Krakow, Poland
Foo Y (Eddy) Liew
Institute of Infection, Immunity and Inflammation College of Medical, Veterinary and Life Sciences University of Glasgow, Glasgow, Scotland, UK
Fernando Martinez Estrada
University of Surrey, Guildford, Surrey, UK
Gioacchino Natoli,
European Institute of Oncology IFOM-IEO, Milan, Italy
Kodi S Ravichandran
Depart. of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, VA, USA
Maria Rescigno
European Institute of Oncology, Department of Experimental Oncology, Milan, Italy
Anna Katharina Simon
Human Immunology Unit, The Weatherall Institute of Molecular Medicine (WIMM), University of Oxford, John Radcliffe Hospital, Oxford, UK
Quentin Sattentau
Sir William Dunn School of Pathology University of Oxford, Oxford, UK
Miguel Soares
Gulbenkian Institute Oeiras, Portugal
Giuseppe (Gio) Teti
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The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*), *Istituto di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italy;* Antonio Di Giacomo, *V Monaldi Hospital, Napoli, Italy;* Tricia Reynolds, *Scientific Secretariat, Scuola Superiore d'Immunologia Ruggero Ceppellini.*

Secretariat: Effe Erre Congressi, Napoli, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Gender Equality and Career Development Committee, International Union of Immunological Societies (IUIS); the Department of Translational Medicine, Federico II University of Naples; The Wellcome Trust; Biolegend; GlaxoSmithKline.

EFIS-EJI Course on "Tumour Immunology: from Tissue Microenvironment to Immunotherapy"

Complesso dei SS. Marcellino e Festo, Università di Napoli "Federico II",
16-18 October, 2017

The Course offered an overview of recent advancements in our knowledge about tumor/host interaction. New avenues for investigation of anti-cancer immunity have recently opened, with enormous translational potential.

The Course Directors

Catherine Sautès-Fridman
Immunopathology Department, Cordeliers Research Centre, Université Paris Descartes, Paris, France
Wolf Fridman
Cancer and Immune Escape Laboratory, Cordeliers Research Centre, Université Paris Descartes, Paris, France
Ennio Carbone
University of Magna Græcia, Catanzaro, Italy

The Faculty

Vincenzo Bronte
Sezione di Immunologia Department of Medicine Verona University Hospital Verona, Italy
Ennio Carbone
University of Magna Græcia, Catanzaro, Italy
Federica Cavallo
Molecular Biotechnology Center, University of Turin, Torino, Italy
Nadine Cerf-Bensussan
Laboratory of Intestinal Immunity, INSERMU1163-Institut Imagine & Université Paris Descartes-Sorbonne Paris Cité, Paris, France
Soldano Ferrone
Department of Surgery Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
Wolf Fridman
Cancer and Immune Escape Laboratory, Cordeliers Research Centre, Université Paris Descartes, Paris, France
Michele Maio
Division of Medical Oncology and Immunotheraqpy, Department pf Oncology, University Hospital of Siena, Siena, Italy
Alberto Mantovani
Istituto Clinico Humanitas, Milan, Italy
Lorenzo Moretta
Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
Dimitrios Mougiakakos
Department of Hematology and Oncology University Hospital of Erlangen- Nuremberg, Nuremberg, Germany
Ugur Sahin
TRON Translational Oncology Johannes Gutenberg-University Mainz, Germany
Catherine Sautès-Fridman
Immunopathology Department, Cordeliers Research Centre, Université Paris Descartes, Paris, France
Hergen Spits
Academic Medical Center University of Amsterdam Amsterdam, Netherlands
Zlatko Trajanoski
Division for Bioinformatics-Biocenter Medical University of Innsbruck Innsbruck, Austria

The Organizing Committee: Silvia Fontana (*President, Scuola Superiore d'Immunologia Ruggero Ceppellini*); Giuseppina Ruggiero, *University if Naples Federico II, Naples, Italy;* Francesca Di Rosa, *Institute of Molecular Biology and Pathology, National Research Council of Italy (CNR), Rome, Italy;* Francesco Colucci, *University of Cambridge, Cambridge, UK.*

Secretariat: Roberta Saioni, Fuori Rotta Eventi & Congressi, Italy.

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Gender Equality and Career Development Committee, International Union of Immunological Societies (IUIS); International Centre for Genetic Engineering and Biotechnology (ICGEB); Université Paris Descartes; Network Italiano per la Bioterapia dei Tumori (NIBIT); Servier; Institute de Recherche Pierre Fabre; Innate Pharma; Dipartimento Medicina Sperimentale e Clinica, University of Magna Græcia, Catanzaro, Italy.

EFIS-EJI Course on "T Cell Memory"

"Osservatorio Cultura Ricerca Formazione Divulgazione" (OCRFD) Congress Center,
Italian National Research Council (CNR),
Anacapri, Capri island, near Naples, 12-15 October 2018

The Course offered an overview of key cellular and molecular signals required for a durable T cell response, focussing on emerging themes in the field. Fundamental questions and translational implications were discussed. Participants had plenty of opportunity for scientific interactions and networking in a friendly atmosphere. Course was held in the charming CNR congress center in Anacapri.

The Course Directors

Francesca Di Rosa
Institute of Molecular Biology and Pathology, National Research Council of Italy (CNR), Rome, Italy
Stephen Schoenberger
La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

The Faculty

Vincenzo Barnaba
Department of Internal Medicine and Medical Specialties, Sapienza University of Rome, Italy
Francesca Di Rosa
Institute of Molecular Biology and Pathology, National Research Council of Italy (CNR), Rome, Italy
Peter Katsikis
Department of Immunology, Erasmus University Medical Center, Rotterdam, The Netherlands
David Masopust
Department of Microbiology and Immunology, University of Minnesota, Minneapolis, MN, USA
Polly Matzinger
National Institutes of Health (NIH), Bethesda, MD, USA
Luigia Pace
Italian Institute for Genomic Medicine, Turin, Italy
Stephen Schoenberger
La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA
Rene van Lier
Sanquin Blood Supply Foundation, Amsterdam, The Netherlands
Andrew Weinberg
Laboratory of Basic Immunology, Earle A. Chiles Research Institute Providence Heath & Services, Portland, OR, USA
Dietmar Zehn
The Technical University of Munich, Freising, Germany

The Organizing Committee: Silvia Fontana, *President, Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy;* Giuseppe Matarese & Giuseppina Ruggiero, *Università di Napoli Federico II, Napoli, Italy* Ennio Carbone, *Magna Grecia University, Catanzaro;* Francesco Colucci, *University of Cambridge, Cambridge, UK.*

Secretariat: Joanna Cyran, *Coordinator, Scuola Superiore d'Immunologia Ruggero Ceppellini;* Roberta Saioni, *Fuori Rotta Eventi e Congressi, Italy.*

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); Gender Equality and Career Development Committee, International Union of Immunological Societies (IUIS); the Andrew and Mary Weinberg Foundation; the Immunotherapy Foundation; the Company of Biologists; ACEA Biosciences; GlaxoSmithKline; M&M Biotech; Euroclone; Biotechne; Biolegend; Miltenyi Biotech; Agilent Technologies; Aurogene; Tema.

EFIS-EJI Course on "Microbes, Immunity and Cancer"

"Osservatorio Cultura Ricerca Formazione Divulgazione" (OCRFD) Congress Center,
Italian National Research Council (CNR),
Anacapri, Capri island, near Naples, 8-10 October 2019

World leaders discussed fundamental and clinically relevant aspects of the interactions between the microbiome and the immune system. These interactions influence both how certain cancers develop and how cancer patients respond to new immunotherapies.

The Course Directors

Francesco Colucci
University of Cambridge, Cambridge, UK
Ennio Carbone
University of Magna Græcia, Catanzaro, Italy
Guido Kroemer
Centre de Recherche des Cordiliers, National Cancer Institute, Université Paris Descartes, Paris, France
Giorgio Trinchieri
National Institute of Health, Bethesda, MD, USA
Laurence Zitvogel
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The Faculty

Petter Brodin
Karolinska Institutet, Stockholm, Sweden, Karolinska University Hospital, Stockholm, Sweden
Jolande De Vries
Radboud University Medical Center, Nijmegen, The Netherlands, Nijmegen, The Netherlands
Sebastian Kobold
Klinikum der Universität München, LMU Munich, Germany
Guido Kroemer
Centre de Recherche des Cordeliers, Paris, France, Gustave Roussy Comprehensive Cancer Center, Paris, France
Nicola Segata
Erasmus University, University of Trento, Trento, Italy
Giorgio Trinchieri
Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, USA
Laurence Zitvogel
Gustave Roussy Comprehensive Cancer Center, Paris, France

The Organizing Committee: Silvia Fontana e Riccardo Zappacosta, *Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy;* Giuseppina Ruggiero, *Università di Napoli Federico II, Napoli, Italy.*

Secretariat: Roberta Saioni, *Fuori Rotta Eventi e Congressi, Italy.*

Sponsorships: European Federation of Immunological Societies (EFIS)- The European Journal of Immunology (EJI); The European Academy of Tumor Immunology (EATI); Gender Equality and Career Development Committee, International Union of Immunological Societies (IUIS); The Department of Experimental and Clinic Medicine (DMSC), University of Catanzaro, Catanzaro, Italy.

LEVEL 3 COURSES

Level 3 Courses are practical, laboratory courses, dealing with recent techniques, and devised for small groups of graduates.

"Molecular Analysis of T Cell Repertoires by CDR3 Length Heterogeneity"

Federico II University Medical School, Naples, Italy,
8-10 May 1998

This laboratory course was aimed to practically show, to a limited number of students, the molecular analysis of T-cell receptor (TCR) reportoire, based on the assessment of the length heterogeneity of CDR3 regions, specifically involved in antigen recognition. The course was inaugurated on May 8, 1998, by a lecture session dealing with the significance of the variations of the TCR repertoires in human pathology and the diagnostic relevance of molecular approaches to such studies.

The Course Director

Jack Gorski,
Blood Research Institute, BloodCenter of Wisconsin, Milwaukee, WI, USA

The Faculty

Alfredo Ciccodicola
International Insitute of Genetics and Biophysics, Italian National Research Council (CNR), Naples, Italy
Raffaele De Palma
Laboratorio di Medicina Molecolare-IRCCS Fondazione S. Maugeri, Pavia
Dipartimento di Internistica Clinica e Sperimentale-Seconda Università di Napoli, Napoli
Claudia Giachino
Laboratorio di Medicina Molecolare-IRCCS Fondazione S. Maugeri, Pavia
Jack Gorski
Blood Research Institute, Blood Center of Southeastern Wisconsin-Medical Complex of Wisconsin, Milwaukee, WI, USA
Philippe Kourilsky
Pasteur Institute, Paris, France
Antonio Lanzavecchia
Basel Institute for Immunology, Basel, Switzerland

The laboratory activity took piace from 9 to 10 May, 1998, at the Genome Research and Sequencing Laboratory, Servizio di Tecnologie Biomolecolari, Area di Ricerca del CNR, Naples and included amplification of the V-D-J regions of TCR gene transcripts by primers specific to each family of such molecules, sequence gel visualization of the obtained products and computer analysis of the data by especially devised softwares. Experiments were carried out under the supervision of Laleh Ansari (Milwaukee), Alfredo Ciccodicola (Naples), Raffaele De Palma (Naples & Pavia), Anna Maria Masci (Naples), Giuliana Soldati (Naples), Maryam Yassai (Milwaukee)

The Organizing Committee: Alfredo Ciccodicola, *International Insitute of Genetics and Biophysics, Italian National Research Council (CNR), Naples, Italy;* Raffaele De Palma & Luigi Racioppi, *Università di Napoli Federico II;* Guido Sacerdoti, *Seconda Università di Napoli.*

Secretariat: Ceppellini School Director's Office, Napoli, Italy.

Sponsorships: The International Institute of Genetics and Biophysics and Area di Ricerca, CNR Naples; The Post-Graduate School of Allergology and Clinical Immunology of the Seconda Università di Napoli, Naples, Italy; Perkin Elmer Italia SpA, Milan, Italy.

OTHER EVENTS TILL 2006

Inaugural ceremony of the "Ruggero Ceppellini Advanced School of Immunology"

Palazzo Serra di Cassano, Naples,
October 11, 1992

The School's inaugural ceremony was held at the seat of the Istituto Italiano per gli Studi Filosofici, on the occasion of the School's first course, dealing with the immunology of bone marrow transplantation. Talks entitled as follows were delivered in that memorable session:

"Plato, Jerne, Ceppellini: Speculation and Experiment in Immunology"
by Jan Klein
Max Planck Institute for Biology, Tubingen, Germany

"The Ruggero Ceppellini Legacy"
by Giovanni B Ferrara
Federico II University of Naples, Italy

"Immunology: Basic Principles and Challenges"
by Alfred Nisonoff
Brandeis University,Waltham, MA, USA

"The Advanced School of Immunology: Aims and Ideals"
by Serafino Zappacosta & Antonio Di Giacomo
SZ: Federico II University of Naples, Italy; AD: Colli Monaldi Hospital, Naples, Italy

"Concluding Address"
by Gerardo Marotta
Istituto Italiano per gli Studi Filosofici, Naples, Italy

Conference "Migration Flows and Emerging Pathologies: The Role of Immunology"

Palazzo Serra di Cassano, Naples, April 29, 2005

A Conference held on the Occasion of the European Day of Immunology, coorganised with the Italian Society for Immunology, Clinical Immunology and Allergology (SIICA).

Chairman

Serafino Zappacosta
Cattedra di Immunologia, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II, Napoli, Italy

Invited Speakers

Helmut Hahn
Institut für Medizinische Mikrobiologie und Infektionsimmunologie, Freie Universität Berlin, Berlin, Germany
Eleanor M Riley
Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK
Stephan Becker
Institut für Virologie, Marburg, Germany
Giuseppe Scala, *Università Magna Græcia, Catanzaro, Italy*

Symposium "Interface between Innate and Adaptive Immunity: Conversation between Tissues and T Cells"

Ischia, near Naples,
April 27, 2004

A Ceppellini School Plenary Symposium within the 3rd National Conference of the Italian Society for Immunology, Clinical Immunology and Allergology (SIICA).

Invited Speakers

Polly Matzinger
Laboratory for Cellular and Molecular Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA
Daniele D'Ambrosio
BioXell, Milan, Italy

Workshop "Meeting the Challenges of Clinical Organ Transplantation"

Azienda Ospedaliera V Monaldi, Naples, June 16, 2000

A Workshop on the biological bases and the clinical perspectives of organ transplantation, in collaboration with the Azienda Ospedaliera V Monaldi, under the auspices of the Second University of Naples and of the Health Service of the Campania Regional Government.

Chairmen

Robert Lechler
Medical Research Council Centre for Transplantation, King's College London, London, UK
Maurizio Cotrufo
Seconda Università di Napoli, Ospedale Mondaldi, Napoli, Italy

Invited Speakers

Maurizio Cotrufo
Seconda Università di Napoli, Ospedale Mondaldi, Napoli, Italy
Andrew JT George
Section of Molecular Immunology, Department of Medicine, Imperial College London, Hammersmith Hospital, London, UK
Robert Lechler
Medical Research Council Centre for Transplantation, King's College London, London, UK
Anthony N Warrens
Renal and Transplantation Medicine, Barts and The London School of Medicine and Dentistry , Queen Mary University of London , London , UK.
Kathryn Wood
Nuffield Department of Surgery, University of Oxford, Oxford, UK
Serafino Zappacosta
Cattedra di Immunologia, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II, Napoli, Italy

Conference "Emergence of Infectious Diseases: An Evolutionary Perspective"

Palazzo Serra di Cassano, Naples,
27-29 May 1998

A Conference aimed at a reappraisal of the biological and social significance of emerging and reemerging infectious diseases. The Conference included two panel discussions, one on "Socioeconomic and Historical Aspects of Infection in Developing vs Developed Countries" (A. Caprioli, Rome, Italy; P. Conforti, Rome, Italy; D. Greco, Rome, Italy; J. A. Louis, Geneva and Epalinges, Switzerland; I. Luzzi, Rome, Italy), and the other on "New Views on HIV Infection" (M. Clerici, Milan, Italy; C. F. Perno, Rome, Italy; O. Perrella, Naples, Italy). Under the patronage of: The World Health Organization; The Health Minister of the Italian Republic; The Istituto Superiore di Sanità, Rome, Italy; The Major of Naples; The Provincia di Napoli.

Chairmen

Jan Klein
Max Planck Institute for Biology, Tubingen, Germany
Serafino Zappacosta
Cattedra di Immunologia, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II, Napoli, Italy

Invited Speakers

Martin Achtman
Max PLanck Institut for Molecular Genetics, Berlin, Germany
Donato Greco
Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanità, Rome, Italy
Eduardo A Groisman
Howard Hughes Medical Institute, Washington University School of Medicine, St Louis, MO, USA
Sunetra Gupta
The Wellcome Trust Centre for the Epidemiology of Infectious Diseases, University of Oxford, Oxford, UK
Helmut Hahn
Institut für Medizinische Mikrobiologie und Infektionsimmunologie, Freie Universität Berlin, Berlin, Germany
Adrian V S Hill
Institute of Molecular Medicine, John Radcliffe Hospital, Oxford University, Oxford, UK
Jonathan C Howard
Institute for Genetics, University of Cologne, Germany
Jan Klein
Max Planck Institute for Biology, Tubingen, Germany
Jack A Louis
World Health Organization, Geneva and University of Lausanne, Epalinges, Swizterland
Andrew J S Macpherson
Institute of Experimental Immunology, University Hospital Zurich, Zurich, Swizterland
Stephen M Ostroff
National Center for Infectious Diseases, Center for Disease Control and Prevention, Atlanta, GA, USA
Rino Rappuoli
Istituto Ricerche Immunobiologiche Siena, Chiron SpA, Siena, Italy
Margaret A Riley
Yale University, New Haven, CT, USA
Thomas S Whittam
The Pennsylvania State University, PA, USA
Serafino Zappacosta
Cattedra di Immunologia, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli Federico II, Napoli, Italy

The Organizing Committee: Antonio Di Giacono Azienda Ospedaliera V Monaldi, Napoli, Italy, Donato Greco, Istituto Superiore di Sanità, Rome, Italy; Jack A Louis, World Health Organization, Geneva and University of Lausanne, Epalinges, Swizterland; Giuseppina Ruggiero, Università di Napoli Federico II, Napoli, Italy.

Secretariat: Effe Erre Congressi, Napoli, Italy.

SERAFINO ZAPPACOSTA CONFERENCES

These conferences were initiated in 2007, in honor of Serafino Zappacosta (1935-2006). Since 2010, most of these events have been held in the newly inaugurated "Serafino Zappacosta" Auditorium of the Federico II University of Naples.

I Serafino Zappacosta Conference

"Xeno-transplantation: biological advances, clinical possibilities, philosophical and ethical concerns"

Invited Speaker: Robert J. Lechler
King's College London, Guy's Hospital, London, UK

Gerardo Marotta, chairman of the Istituto Italiano per gli Studi Filosofici, introduced this first conference. He discussed the basis of the very intriguing interplay between Science and Philosophy that the long-standing collaboration between the Scuola Superiore d'Immunologia Ruggero Ceppellini and the Istituto Italiano per gli Studi Filosofici has been proposing to the scientific community since many years. The invited speaker gave an intriguing overview on key issues in xenotransplantation.

Palazzo Serra di Cassano, Istituto Italiano per gli Studi Filosofici, Naples, February 1st, 2007

II Serafino Zappacosta Conference

"1908-2008, Science and culture in Naples: the Metchnikoff heritage a century after the Nobel Prize"

Invited Speaker: Helmut Hahn
Berlin Medical Association and Koch-Metchnikoff Forum, Berlin, Germany

"Phagocytosis 100 years later: Imaging proteins, lipids and charges"

Invited Speaker: Sergio Grinstein
Hospital for Sick Children, Toronto, Canada

This conference proposed a fruitful discussion on the role of the Stazione Zoologica Anton Dohrn, a pioneer neapolitan institution, for the development of Metchnikoff theories on cell-mediated processes in immune recognition.

Stazione Zoologica Anton Dohrn, Naples, June 16, 2008

III Serafino Zappacosta Conference

"Small RNAs, transcription and epigenetic modifications"

Invited Speaker: V. Enrico Avvedimento
Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli, Federico II, Napoli

"Proteins and microRNAs controlling NK cell activity"

Invited Speaker: Ofer Mandelboim
The Hebrew University of Jerusalem, Jerusalem, Israel

This conference was focused on the emerging interplay between small interfering RNAs and immune-regulation.

Palazzo Serra di Cassano, Istituto Italiano per gli Studi Filosofici, Naples, June 4, 2009

IV Serafino Zappacosta Conference

"Allelic exclusion in the immune system"

Invited Speaker: Yehudit Bergman
Department of Developmental Biology and Cancer Research, The Hebrew University Medical School, Jerusalem, Israel

This conference focused on the role of genetic processes in the generation and development of adaptive humoral immune response.

Aula "Serafino Zappacosta", Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli "Federico II", June 30, 2010

V Serafino Zappacosta Conference

"Paroxysmal Nocturnal Haemoglobinuria: Stem cells, Complement, Autoimmunity"

Invited Speaker: Lucio Luzzatto
Università di Firenze, Istituto Toscano Tumori, Firenze

This conference discussed the intriguing involvement of autoimmune selection processes in the emergence and dominance of Bone Marrow defective progenitors in the pathogenesis of Paroxysmal Nocturnal Haemoglobinuria.

Aula "Serafino Zappacosta", Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli "Federico II", January 27, 2012

VI Serafino Zappacosta Conference

"The immune contexture of human tumours"

Invited Speaker: Catherine Sautès-Fridman
Immunology, Cancer and Inflammation Department, Université Paris Descartes, Paris, France

This conference highlighted the relevance of immune contexture in the priming and differentiation of an effective anti-tumor immune response. It also addressed the prognostic potential of the analysis of immune infiltrates in solid tumors.

Aula "Serafino Zappacosta", Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli "Federico II", September 12, 2014

VII Serafino Zappacosta Conference

"What triggers an immune response?"

Invited Speaker: Polly Matzinger
Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, USA

Ennio Carbone & Giuseppe Matarese, two past students of prof Zappacosta and current members of the Board of Directors of the Ceppellini School of Immunology introduced this conference. They highlighted that the Ceppellini School represents a precious heritage after the death of Serafino Zappacosta. The invited speaker, a world leader in immunology, offered her provocative ideas on immune tolerance and discussed the reasoning behind them.

Complesso dei SS. Marcellino e Festo, Università di Napoli "Federico II", September 28, 2016

VIII Serafino Zappacosta Conference

Epigenetic cancer immuno-modeling to improve the efficacy of checkpoint-based immunotherapy

Invited Speaker: Michele Maio
Center for Immuno-Oncology, Medical Oncology and Immunotherapy, Siena University Hospital, Siena

The invited speaker gave an historical overview on Immuno-therapy, highlighting that recent advancements in this field have provided (by now) well-established therapeutic tools, powerfully improving clinical management of human tumors.

Auditorium "Gaetano Salvatore", Università di Napoli "Federico II", March 12, 2019

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