Macrophages: Development and Tissue Specialization

Chen Varol,[1] Alexander Mildner,[2] and Steffen Jung[2]

[1]The Research Center for Digestive Tract and Liver Diseases, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 64239, Israel

[2]Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel; email: s.jung@weizmann.ac.il

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Abstract

Macrophages are myeloid immune cells that are strategically positioned throughout the body tissues, where they ingest and degrade dead cells, debris, and foreign material and orchestrate inflammatory processes. Here we review two major recent paradigm shifts in our understanding of tissue macrophage biology. The first is the realization that most tissue-resident macrophages are established prenatally and maintained through adulthood by longevity and self-renewal. Their generation and maintenance are thus independent from ongoing hematopoiesis, although the cells can be complemented by adult monocyte-derived macrophages. Second, aside from being immune sentinels, tissue macrophages form integral components of their host tissue. This entails their specialization in response to local environmental cues to contribute to the development and specific function of their tissue of residence. Factors that govern tissue macrophage specialization are emerging. Moreover, tissue specialization is reflected in discrete gene expression profiles of macrophages, as well as epigenetic signatures reporting actual and potential enhancer usage.

INTRODUCTION

Macrophages are myeloid immune cells that are characterized by avid phagocytosis. Befittingly, Ilya (Elie) Metchnikoff, the father of cellular immunology, hence referred to these cells as "the big eaters" in Greek (1). With extraordinary intuition, Metchnikoff, a trained comparative zoologist, foresaw the fascinating biology of macrophages and their central role in health and disease states and was rightfully awarded a Nobel prize. Using intravital microscopy to visualize macrophage swarms in pricked starfish larvae and infected water fleas, he noted the striking similarities of invertebrate phagocytosis and vertebrate blood cells. He proposed that macrophages had originally evolved to regulate development through phagocytosis of unwanted or obsolete cells, such as in the pruning of digits and frog morphogenesis. Activities of these professional phagocytes might then have been recruited for innate immune effector functions, including pathogen clearance. Importantly, beyond regulating development and controlling pathogens, Metchnikoff also recognized that macrophages play a major role in injury repair and appreciated their contribution to sterile physiological inflammation (1). He thereby anticipated the role of inflammation and macrophages in homeostatic maintenance processes of a healthy organism, very much in line with currently held views (2, 3).

Below we discuss our view of the recent paradigm shifts in our understanding of tissue macrophages, including distinct developmental pathways and the emerging theme of tissue specialization. In light of the many recent excellent and comprehensive reviews on macrophages (4–8), we focus here on macrophage functions in a healthy organism and the underlying design principles of this cellular network and refer to examples mainly derived from mouse models in support of our arguments.

DEVELOPMENTAL ORIGINS OF TISSUE MACROPHAGES

Macrophages were classified as part of the mononuclear phagocyte system established by van Furth & Cohn (9, 10), along with monocytes and dendritic cells (DCs), which were included upon their discovery by Steinman & Cohn (11) in the mid-1970s (12, 13). The mononuclear phagocyte concept held that tissue-resident macrophages in adulthood rely on replenishment by bone marrow (BM)-derived blood monocytes (10). However, the notion of an inherent developmental link between tissue macrophages and blood monocytes has been disputed throughout the years (14). The long-term persistence of adult tissue macrophages relying on self-renewal is well established. Moreover, certain embryonic macrophage populations are established before the emergence of circulating monocytes. Finally, monocytopenic animals display seemingly normal tissue macrophage compartments, and the number of tissue-resident macrophages is also largely unaffected in human patients suffering from monocytopenia (see 14). Collectively, these findings motivated fate mapping experiments to address macrophage origins. These experiments revealed the major alternative, nonmonocytic origin of these cells, and thereby led to the revision of an obligatory steady-state monocyte-macrophage axis.

The revised concept of the mononuclear phagocyte system, although still mainly based on data from the mouse, now accommodates the existence of tissue macrophages of two distinct origins. With regard to the first, the majority of macrophages that reside in healthy tissue are established prenatally. These cellular compartments self-maintain locally independently from each other within their tissue of residence and are self-sufficient and independent from further hematopoietic input. With regard to the second, macrophages can develop in adulthood from tissue-infiltrating monocytes. This pathway is associated with pathological, but also homeostatic, inflammatory reactions. Monocyte-derived macrophages generally display limited life spans,

although exceptions have been reported. Embryonic- and adult-derived macrophages can coexist in given tissues, and their respective abundance likely reflects the nature and history of their tissue of residence. Whether embryonic- and adult-derived macrophages differ with respect to functional contributions in homeostasis or following challenge remains to be shown, however, and is a key question with major clinical relevance.

Embryonic Macrophages

Macrophages are mesoderm-derived hematopoietic cells. Embryonic hematopoiesis occurs in independent waves and shifts in a temporally and spatially coordinated manner between organs. In rodents, the first wave is generally considered to be transient and starts at embryonic day 7 (E7) in the blood islands of the extraembryonic yolk sac. It produces primitive nucleated erythrocytes and myeloid cells but not lymphocytes (15, 16). A second wave is initiated within the hematogenic endothelium of the aorta-gonad-mesonephros (AGM) of the embryo at E10.5 (17, 18). This so-called definitive hematopoiesis yields enucleated erythrocytes and generates a limited number of stem cells that harbor full multihematopoietic lineage potential. Mostly AGM-derived, but also yolk sac–derived, hematopoietic progenitor cells then colonize the fetal liver (19), which transiently becomes the predominant embryonic site of hematopoiesis. Postnatal murine primary hematopoietic organs, such as the spleen and BM, are colonized by fetal liver–derived hematopoietic progenitors via the circulatory system and eventually host hematopoietic stem cells (HSCs) in specialized niches (17, 18).

Primitive macrophages appear in the yolk sac blood islands at E8.5/E9.0 and are generated without monocytic intermediates. These cells spread upon the establishment of the blood circulation from the yolk sac and seed the whole embryo (20). Early fetal tissue macrophages retain proliferative potential, and these cells are likely in charge of clearing debris associated with developmental tissue remodeling, the pruning of cellular structures and cells, and their functioning as chaperones in vascularization (21–23). Accordingly, mouse mutants lacking macrophages during embryonic development, for instance, owing to PU.1 (also known as SPI1) or colony-stimulating factor 1 receptor (CSF-1R; also known as M-CSFR or CD115) deficiencies, display perinatal mortality and growth retardation (24, 25). Once definitive hematopoiesis sets in, it becomes the major source of embryonic hematopoiesis, including the generation of fetal monocytes. The latter phenotypically resemble adult monocytes, including in their expression of CSF-1R, although the generation of fetal monocytes seems independent of the known CSF-1R ligands CSF-1 and IL-34 (26).

Adult Tissue Macrophages of Embryonic Origin

It has long remained unclear whether embryonic macrophages persist into adulthood and, if so, whether these cells, and thus primitive hematopoiesis, significantly contribute to adult tissue macrophage compartments. Analysis of mutant mice, which harbor a selective impairment of definitive, but not primitive, hematopoiesis, recently established that yolk sac–derived cells can indeed be part of tissue-resident macrophage populations in the adult (27). Specifically, these experiments took advantage of the fact that the transcription factor Myb is dispensable for yolk sac myelopoiesis but is required for definitive HSC-derived hematopoiesis (28, 29). Interestingly, Myb-deficient embryos harbor normal tissue-resident macrophages in multiple organs, including the liver [Kupffer cells (KCs)], epidermis [Langerhans cells (LCs)], and brain (microglia) (27). Of note, these data are consistent with observations in Myb mutant zebrafish, which also display normal postnatal tissue-resident macrophage populations in the absence of definitive hematopoiesis (30).

The persistence of embryonic macrophages into adulthood was also documented by fate mapping studies involving Cre-loxP approaches. The combination of a Cre recombinase and conditional floxed reporter transgenes, driven by the ubiquitous expressed Rosa26 (R26) promoter, allows the introduction of permanent genetic marks into defined cell populations and their subsequent tracing over time. Analysis of reporter animals that harbor a green fluorescent protein reporter gene insertion in the locus of the chemokine receptor CX_3CR1 established prominent CX_3CR1 expression in the mononuclear phagocyte compartment (31). This includes myeloid precursors, such as primitive macrophages (16), macrophage (monocyte)/dendritic cell precursors (MDPs) (32), and common monocyte progenitors (cMOPs) (33), as well as fetal and adult monocytes (26, 34). With some exceptions (e.g., the microglia and intestinal macrophages), tissue macrophages, including liver KCs and lung macrophages, silence CX_3CR1 expression during development or tissue specification (35). Owing to their derivation from CX_3CR1-expressing precursors, these cells still activated the reporter in CX_3CR1^{Cre}:R26-YFP mice that display constitutive Cre activity in CX_3CR1^{+} cells. Interestingly, postnatal drug-induced activation of Cre activity in CX_3CR1^{CreER}:R26-YFP mice did not result in the expression of the label in these CX_3CR1^{-} macrophages (35). This finding established that most tissue macrophage compartments are generated before birth and, at least in the absence of challenge, are quantitatively maintained in adulthood through longevity or limited self-renewal.

Other fate mapping studies have aimed at dissecting the relative contribution of the yolk sac and fetal liver to specific adult tissue macrophage populations. Experiments based on inducible CreER transgene expression driven by one of the promoters of the runt-related transcription factor 1 (Runx1) substantiated and extended the earlier notion that brain macrophages (i.e., microglia) are established in the embryo (36, 37). Of note, Runx1 expression is restricted to the extra-embryonic yolk sac between E6.5 and E8 (38), and $Runx1^{CreER}$ mice therefore allowed for time-window-restricted pulse labeling of the myeloid precursors to further distinguish the contributions of cells residing in the yolk sac and those in the fetal liver. With the notable exception of microglia that originate exclusively from yolk sac–derived cells, it was found that adult tissue macrophage populations, including those of the lung, dermis, and spleen, receive considerable input from fetal liver–resident precursors (37). Similarly, genetic fate mapping involving $Flt3^{Cre}$ mice showed that both yolk sac–derived and fetal liver–derived progenitors give rise to heart-resident, cardiac macrophages (39). This notion is further supported by the finding that adult LCs mainly originate from fetal liver–derived monocytes that seed the developing skin mesenchyme around E14.5 and replace the majority of yolk sac–derived LCs (26). Moreover, adult lung alveolar macrophages were shown to originate from fetal liver monocytes in a CSF-2 (GM-CSF)-governed pathway (40).

The independence of adult tissue macrophage compartments from monocyte input is also supported by parabiosis studies involving mice whose circulation is surgically joined (41). Lymphocytes of the parabionts reach with time equilibrium in the joined circulation, and monocyte compartments exhibit considerable chimerism. If these monocytes were differentiating into tissue macrophages in the steady state, the tissue macrophages would be expected to display similar chimerism in the tissue of the partner parabiont. However, tissue macrophages failed to equilibrate even after 5 months of parabiosis, suggesting the absence of an ongoing steady-state contribution of BM-derived cells to adult tissue macrophage compartments (41).

Collectively, these data establish that most tissue macrophages originate before birth and subsequently self-maintain through longevity with limited proliferation. Contributions of yolk sac– versus fetal liver–derived precursors, however, vary between tissue macrophage compartments (**Figure 1**). Specifically, and potentially related to their unique seclusion behind the blood-brain barrier (BBB), microglia have emerged as a population that originates exclusively from yolk sac–derived hematopoietic progenitors, with little, if any, contribution from hematopoietic

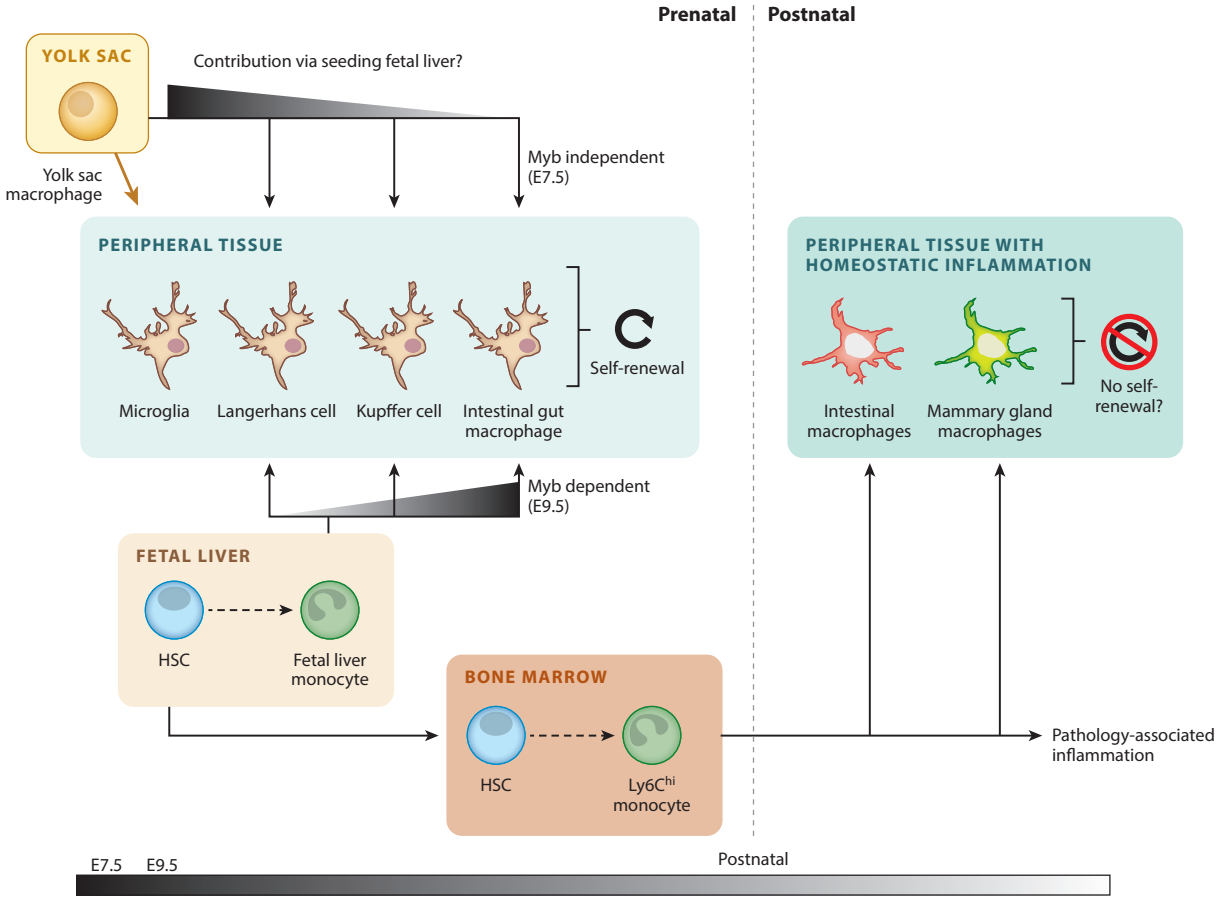

Figure 1

Development of tissue-resident macrophages. Primitive macrophages in the yolk sac appear around embryonic day 7 (E7) and disseminate following the establishment of the blood circulation (E9.5) throughout embryonic tissues. This primitive hematopoiesis is independent of the transcription factor Myb. With the colonization of the fetal liver by aorta-gonad-mesonephros-derived hematopoietic stem cells (HSCs) around E10.5, the definitive hematopoiesis is initiated in an Myb-dependent manner and generates all major hematopoietic lineages, including monocytes. Fetal liver monocytes infiltrate peripheral tissues except for the central nervous system and give rise to tissue-resident macrophages, which mostly coexist but can progressively outcompete yolk sac–derived tissue macrophages. Yolk sac–derived and fetal liver–derived macrophages are characterized by longevity and self-renewal. During adulthood, Ly6C^{hi} monocytes can give rise to relatively short-lived, non-self-renewing tissue-resident macrophages in organs displaying homeostatic inflammatory conditions, such as the intestine, the remodeling mammary gland, and the heart.

progenitors arising later in embryonic development (42, 43). In contrast, other tissue macrophage compartments that have been studied so far seem to be more promiscuous with respect to their origins.

A derivation from yolk sac–resident precursors equals origin from primitive myelopoiesis that is independent of the transcription factor Myb and HSCs. However, the origin of cells arising from the fetal liver is less well defined. This is due to the fact that this organ, which hosts emerging definitive hematopoiesis, is itself being seeded by yolk sac precursors (15, 16). Accordingly, there is now general agreement that most tissue macrophages are embryo derived and that these

compartments are maintained in adulthood without input from BM-derived cells. However, the exact extent of the differential contributions of primitive and definitive hematopoiesis to these embryo-derived adult tissue macrophage compartments in the adult remains under debate (**Figure 1**). For instance, it has been proposed that all tissue macrophages might form a Myb- and consequently HSC-independent lineage, whose establishment is independent of a monocytic intermediate (44). Alternatively, certain adult tissue macrophages might arise from early definitive hematopoiesis that commences in the fetal liver and involves the generation of fetal monocytes (14). A main distinctive feature of these two scenarios is that the generation of embryonic macrophages according to the latter route involves a monocytic intermediate and is hence more like the inflammation-associated adult pathway (reviewed below).

Of note, adult BM does contain precursor cells that can give rise to bona fide long-lived tissue macrophages, as shown for microglia and LCs (45, 46). The available evidence, however, suggests that these precursor cells are distinct from monocytes, which only transiently seed the respective tissue (46). The identity of the non-monocytic cells requires further investigation. Importantly, it remains unclear whether adult BM retains Myb-independent, yolk sac–derived cells with precursor potential that could seed tissues with macrophages. The identification of such cells could have major clinical relevance for cell therapies.

In summary, it is now widely accepted that adult tissue macrophages are established before birth. The exact contributions of Myb-independent myelopoiesis and definitive Myb-dependent hematopoiesis to adult tissue macrophage compartments, other than the microglia, remain to be established. Finally, it has to be clarified whether Myb-dependent and -independent origins of tissue macrophages have functional implications.

Postnatally Generated Tissue Macrophages

The assumption that macrophages derive from circulating monocytes was based on the classical observation that in pathological settings, monocytes give rise to macrophages (10). Monocytes and, in particular, the Ly6C^{hi} monocyte subset (see below) exhibit a short half-life, much like neutrophils (35). This is consistent with the assumption that these cells constitute a precursor reservoir for tissue-resident mononuclear phagocytes. A BM origin for tissue macrophages was further supported by transplantation experiments showing near-complete reconstitution of tissue macrophage populations, with the exception of LCs and microglial cells (41). As mentioned above, it remains unclear whether the repopulation involves monocytes or earlier, yet to be defined, BM-resident precursors. The monocytic origin of tissue macrophages became a foundational dogma of the mononuclear phagocyte concept, as introduced by van Furth and colleagues (10). It is, however, now firmly established that classical DCs rely on dedicated precursors for their maintenance (12) and that most tissue macrophages are of embryonic origin and are independent of monocyte input (14, 44). Conversely, monocytes and their descendants have emerged as a third, highly plastic and dynamic cellular system that can complement the classical tissue-resident mononuclear phagocyte compartment on demand (47) (**Figure 2**). Of note, contributions of these monocyte-derived cells to various physiological processes can include activities associated with classical tissue macrophages and DCs, including migration to lymph nodes, antigen presentation, and bactericidal activity (47, 48). Monocytes, however, also likely function in their own right as short-lived effector cells in tissues and contribute specific activities that have yet to be better defined; emerging data suggest, for example, that monocytes can promote angiogenesis and arteriogenesis (49). Finally, it was recently proposed that Ly6C^{hi} monocytes persist in the steady state without commitment toward macrophage or DC-like fates and might contribute to antigen transport to lymph nodes (50) (**Figure 3**).

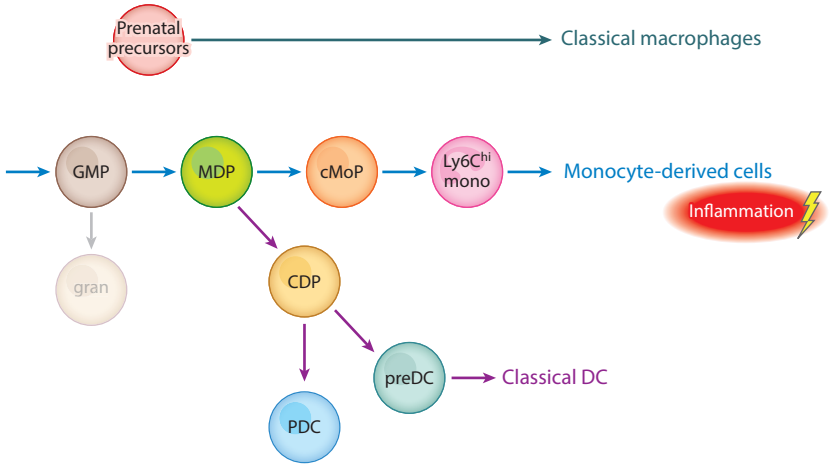

Figure 2

Schematic illustration of the tripartite structure of the mononuclear phagocyte system. Classical macrophages and DCs are residing in tissues, whereas monocytes can be called in from the blood circulation on demand, mostly associated with inflammation. Abbreviations: CDP, common dendritic cell precursor; cMoP, common monocyte progenitor; DC, dendritic cell; GMP, granulocyte/macrophage precursor; MDP, macrophage (monocyte)/dendritic cell precursor; PDC, plasmacytoid DC.

Differential monocyte fates in tissues (i.e., effector monocytes and monocyte-derived DCs or macrophages) likely represent a developmental continuum and might hence resist exact definition (51) (see the sidebar MF and MC: What Is in a Name?). Moreover, in tissue, monocyte-derived cells may acquire phenotypic features that make them difficult to discriminate from resident embryo-derived tissue macrophages. To overcome this technical challenge and nevertheless define the potential differential contributions of these distinct cellular entities, researchers have introduced a number of approaches. Originally, these strategies were based on irradiation and BM transfers (52). However, this approach is problematic, as it causes inflammation and perturbations, such

MF AND MC: WHAT IS IN A NAME?

The intricacies of biology are a major challenge to efforts to classify and establish terminologies that allow for unambiguous identification and communication between researchers. The mononuclear phagocyte system is no exception to this rule, and in particular, recent findings have presented the community with the dilemma of either sticking to historical definitions or considering adjustments. As a major move, it was recently suggested to classify mononuclear phagocytes primarily by their ontogeny and only secondarily by their location, function, and phenotype (51). The authors of this nomenclature proposal suggested that the three branches of the mononuclear phagocyte system (47) should be acknowledged by terming them dendritic cells, macrophages, and monocyte-derived cells. Acceptance of this proposal will likely depend on proof that origins matter (i.e., experimental evidence for functional differences of embryo-derived macrophages and monocyte-derived macrophages). Alternatively, ongoing profiling studies might result in the definition of an epigenetic signature of embryo-derived macrophages. The identification of the latter might currently be hindered by the pronounced heterogeneity of tissue macrophage enhancer landscapes (136). Finally, single-cell transcriptome analysis (211) of macrophage compartments might help to molecularly define embryo-derived macrophages and monocyte-derived macrophages.

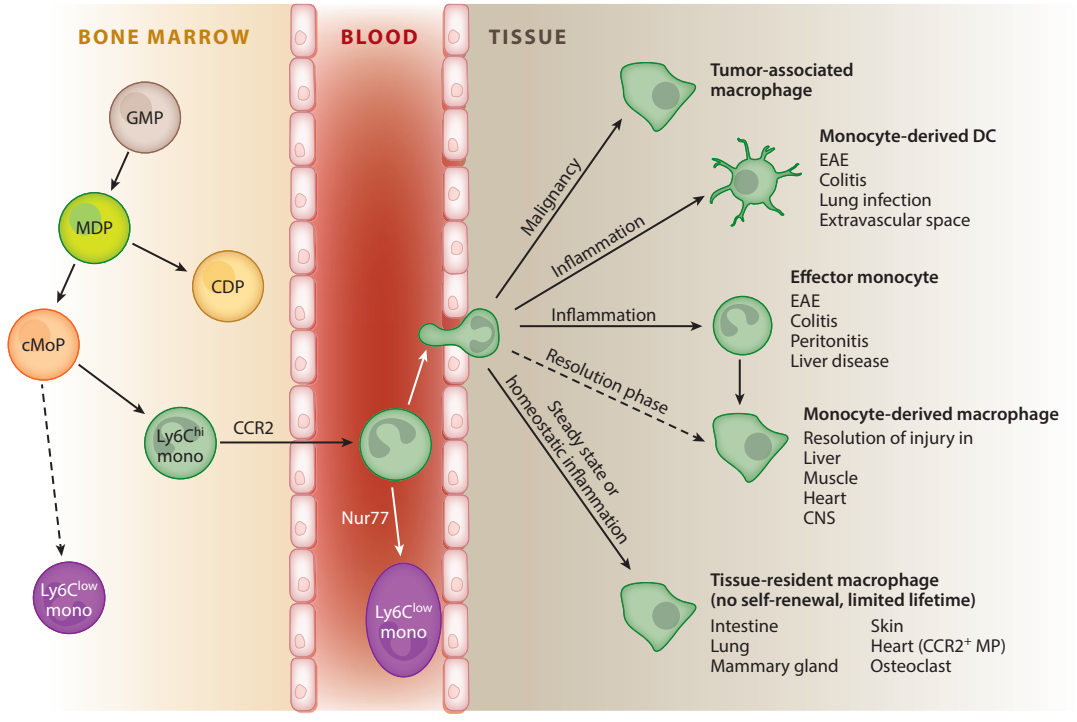

Figure 3

Monocyte fates in tissue and disease settings. Current hematopoietic schemes propose that hematopoietic stem cell–derived GMPs give rise to MDPs, which have lost the potential to differentiate into granulocytes, although a recent publication has challenged the notion of a defined MDP entity (210). MDPs give rise to CDPs and cMoPs (33), which are the direct progenitors of Ly6C^{hi} monocytes. Whether cMoPs are direct precursors of Ly6C^{low} monocytes remains to be shown. Upon their CCR2-dependent extravasation, Ly6C^{hi} monocytes circulate in the blood and are poised to be recruited to sites of inflammation. Inflammatory conditions initiate proinflammatory effector functions in monocytes or promote DC-like functions comprising antigen presentation and migration to LNs. During the resolution phase, monocytes differentiate into restorative macrophages and contribute to tissue repair. Tissue with constant homeostatic inflammation (e.g., the intestinal lamina propria), mechanically stressed tissue (e.g., the heart), or tissue undergoing remodeling allows the development of monocyte-derived macrophages while bypassing the proinflammatory effector monocyte stage. Finally, monocytes also give rise to tumor-associated macrophages that promote tumor development and metastasis. Abbreviations: CDP, common dendritic cell precursor; cMoP, common monocyte progenitor; CNS, central nervous system; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; GMP, granulocyte/macrophage precursor; LN, lymph node; MDP, macrophage (monocyte)/dendritic cell precursor.

as a BBB breakage (53). Moreover, the intravenous transfer of BM cells, including precursors cells, can introduce considerable artifacts (46). Monocytes require CCR2 for their exit from the BM (54), and CCR2 dependence of a given tissue-resident macrophage population can hence serve as an indicator of its monocyte origin (35, 55). Moreover, the CCR2 promoter has been used to drive a reporter gene to label monocytes and distinguish these cells from tissue-resident macrophages (56, 57). Conversely, fate mapping strategies that activate reporter genes can be used to specifically label embryo-derived cells and distinguish them from rapidly renewed, short-lived, monocyte-derived cells (35, 58). Additionally, the monocyte origin of cells can be established through adoptive transfer experiments (49, 59). Finally, ongoing transcriptome and epigenome profiling efforts might yield markers that will allow more ready discrimination of embryo-derived and adult monocyte-derived cells without means of transgenesis, parabiosis, and irradiation. These

approaches should pave the way for a comprehensive investigation of functional macrophage heterogeneity not only in the mouse, but also in human tissue.

Monocytes: The Precursors

Monocytes can be defined by their location, phenotype, and characteristic bean-shaped nuclei, as well as gene and microRNA expression signatures (60–62). They are present in all vertebrates, with evidence for a parallel cell population in insect hemolymph (63). In mice, monocytes represent 4% of the nucleated circulating leukocytes, with considerable marginal pools in spleen and lungs that can be mobilized on demand (64). Monocyte development in the mouse depends on CSF-1, and a deficiency of the CSF-1 receptor results in severe monocytopenia (25). Monocytes arise during adult hematopoiesis in the BM from cMoPs (33) (**Figure 3**). As opposed to earlier precursors, such as MDPs (65), these cMoPs have lost the potential to give rise to plasmacytoid DCs and classical DCs.

Early studies established the existence of monocyte subsets in human blood (66). $CD115^+$ mouse monocyte subsets are now divided into $Ly6C^{hi}$ monocytes (also defined as $CX_3CR1^{int}CCR2^+CD62L^+CD43^{low}$) and $Ly6C^{low}$ monocytes ($CX_3CR1^{hi}CCR2^-CD62L^-CD43^{hi}$). Results of gene expression profiling support the alignment of the $Ly6C^{hi}$ and $Ly6C^{low}$ mouse populations with the two main $CD14^+$ and $CD14^{low}CD16^+$ human subsets, respectively (60, 61). Mouse and human monocytes comprise additional distinct transition states, which remain less well characterized in terms of phenotype and function (14). Studies in the mouse have established that both monocyte subsets derive from MDPs and cMoPs, although with distinct kinetics suggesting a precursor-product relationship of $Ly6C^{hi}$ and $Ly6C^{low}$ monocytes (33, 59). Indeed, accumulating evidence indicates that murine blood monocyte subsets represent a developmental sequence in the steady state, with $Ly6C^{hi}$ monocytes differentiating, most likely in the circulation, into $Ly6C^{low}$ cells (14, 35, 59). The generation of $Ly6C^{low}$ cells requires the transcription factor NR4a1 (Nur77) (67). It remains to be shown whether $Ly6C^{low}$ cells can also be generated without a $Ly6C^{hi}$ monocyte intermediate. We note that $Ly6C^{hi}$ monocyte-derived $Ly6C^{low}$ cells could be regarded as differentiated blood-resident macrophages. In line with this view, the prime function of $Ly6C^{low}$ cells seems to be the intravascular survey of endothelial integrity (68, 69) rather than the extravasation and engraftment of tissue. In contrast, $Ly6C^{hi}$ monocytes in mice, and $CD14^+$ monocytes in humans, represent classical monocytes, which are recruited to sites of pathological changes, such as tumors, and inflammation and can act as precursors of monocyte-derived tissue macrophages (**Figure 3**) (see below).

Monocyte-Derived Macrophages in Pathology-Associated Inflammation

Monocyte infiltrates are a prominent feature of inflammation and pathology. Depending on the tissue context that the infiltrating monocytes encounter upon extravasation, they can adopt distinct activities, which can considerably overlap with tissue-resident mononuclear phagocytes. Below we discuss selected key disease scenarios, for which contributions of monocyte-derived macrophages have been analyzed in particular detail, especially when contrasted with that of tissue-resident macrophages. This review focuses mainly on the steady state. For further in-depth discussion and primary references, we hence refer the reader to recent excellent reviews on pathology-associated mononuclear phagocytes (4, 70–72).

Tumors are abundantly populated by macrophages, which were originally thought to partake in the host antitumor response. Many of these tumor-associated macrophages (TAMs) are now known to promote tumor initiation, progression, and metastasis (70). This in part results

from their provision of inflammatory cytokines that generate a chronic inflammatory environment permissive for tumor initiation and promotion. In addition, established tumors condition TAMs to switch from an immunologically active state to a trophic immune-suppressive phenotype that promotes tumor progression and malignancy (70). TAMs also critically contribute to the remodeling of the tumor microenvironment through the expression of proteases, including matrix metalloproteinases and cathepsins (73). In addition, TAMs that are recruited by hypoxia and growth factors orchestrate the so-called angiogenic switch, which results in an increase in vascular density, marking the transition to the malignant state (74, 75). Because most tumors develop during adulthood and, similar to other organs, likely depend on a functional macrophage network, the origin of TAMs is of particular therapeutic interest. Given the prominent monocyte infiltrates associated with most tumors, the majority of TAMs are likely monocyte derived (76). Interestingly, monocyte-derived TAMs that suppress cytotoxic antitumor T cell responses and promote tumor growth in a mammary tumor model depend on a locally triggered Notch signal (77). The monocytic origin might render TAMs uniquely amenable to therapeutic approaches, for instance, by employing genetically manipulated HSCs for targeted transgene expression in myelomonocytic progeny (78). Specifically, the forced expression of IFN-α in TAMs was recently shown to reprogram the tumor microenvironment and inhibit tumor progression in an experimental system (78). This strategy could also be used to interfere with the generation of tumor-promoting TAMs. Similarly, genetic and pharmacological targeting of CSF-1 or its receptor can efficiently reduce TAM content and tumor size in several mouse models and human patients (4). Contributions of tissue-resident embryo-derived macrophages to TAM activities remain less well understood and likely differ depending on the tumor location. Specifically, the well-studied mammary gland tumor model might not be representative, as the resident macrophage population of this postnatally developing tissue is also monocyte derived (79). For more detailed information on TAMs and additional references, we refer the reader to Reference 70 for an excellent recent review.

Monocyte accumulations in the vessel wall characterize the earliest visible lesions of both human and experimental atherosclerosis (80). Specifically, monocytes differentiate in the intima (the innermost layer of the artery) into macrophages that ingest modified lipoproteins via scavenger receptors and secrete inflammatory mediators that can stimulate smooth muscle cell migration and proliferation (80). With time, monocyte-derived macrophages give rise to characteristic lipid-laden macrophages, termed foam cells, and thereby become key constituents of the lipid core of the atherosclerotic plaque. The crucial contribution of monocyte-derived cells is highlighted by the fact that atherogenesis is affected by impairment of Ly6C^{hi} monocyte recruitment, differentiation, or survival, for instance, through deficiencies of the CCR2 or CX_3C axis (81, 82). Monocyte contributions to pathological settings were generally regarded as transient and highly dynamic and were believed to rely on continuous monocyte recruitment. However, it was recently shown that maintenance and expansion of the pool of lesional monocyte-derived macrophages in experimental atheromata can involve the local proliferation of these cells rather than continuous de novo monocyte influx (83).

Brain pathologies can be associated with a breakdown of the BBB and prominent monocyte infiltration into the central nervous system (CNS). CNS entry of monocytes is tightly regulated and requires active recruitment, which involves the CC-chemokine ligand 2 (CCL2)/CCR2 axis. Within the brain context, monocytes give rise to brain macrophages that are difficult to discern from the resident embryo-derived microglia. Emerging data, in particular from the study of the murine multiple sclerosis model experimental autoimmune encephalomyelitis (EAE), have established distinct functional contributions of microglia and monocyte-derived cells (e.g., 84). Specifically, CCR2-deficient animals, and mice depleted of Ly6C^{hi} monocytes, show attenuation

of clinical EAE symptoms (84), establishing monocytes or monocyte-derived cells as disease drivers. Impaired recruitment of these cells might explain why mice that harbor TAK1-deficient microglia, which fail to induce CCL2 expression, are relatively protected from EAE (58). In fact, gene expression profiling recently revealed that during EAE, resident microglia remain inert, whereas infiltrating macrophages are immune activated and express proinflammatory genes, such as IL-1β and TNF-α (57, 85). Moreover, using an advanced imaging approach based on the above-mentioned CCR2-reporter mice, researchers found that monocyte-derived macrophages initiated demyelination, whereas microglia appeared dedicated to the clearance of debris (57). This supports the emerging notion that tissue-resident macrophages, as opposed to recruited cells, are robustly imprinted to partially resist stimuli associated with acute inflammation. Such a scenario, which would be of major relevance for the development of therapeutics, is also supported by studies focusing on the acutely inflamed gut (86, 87) and liver (88). The conclusion that monocyte-derived cells and microglia remain distinct entities during disease progression, and also following recovery, is furthermore in line with results of experiments involving a combination of parabiosis and myeloablation (46). Specifically, monocyte-derived macrophages were found to only transiently seed the brain and not permanently integrate into the brain-resident macrophage compartment. The latter is thus composed of embryo-derived microglial cells before and after the challenge (46).

In accordance with monocyte plasticity, monocyte-derived macrophages can also have beneficial effects, such as during recovery from spinal cord injury, which they support by critical production of IL-10 (89). This is in line with many studies that collectively established the critical importance of monocytes for the resolution phase of inflammation (90). It has been shown in the liver that recruited Ly6C^{hi} monocytes differentiate into short-lived pro-restorative Ly6C^{lo}F4/80^{hi} macrophages that outnumber the resident KC population at the early recovery phase from liver fibrosis (91) and drug-induced liver injury (88). Similar examples were also reported in the healing of other tissue-specific injuries, such as myocardial infarction (92), skeletal muscle (93), spinal cord (89), retina (94), and sterile wounds (95).

Monocyte-Derived Macrophages in Homeostatic Inflammation

Their dwelling in the circulation and extreme functional plasticity make monocytes well suited for rapid recruitment and the performance of acutely required effector functions associated with the initiation of the inflammatory reaction and its subsequent resolution. However, inflammation is not only a feature of pathology, infection, and the disease state. It is also a response to physiological noxious conditions associated with tissue remodeling, as well as mechanical stress (2, 3). Moreover, ongoing tonic low-grade inflammation can by itself be a critical component of tissue homeostasis, as outlined below for the intestine.

The finding that most tissue macrophages are established before birth and are hitherto independent from HSCs and monocytes was in stark contrast to recent demonstrations that intestinal macrophages seem in their entirety to be monocyte derived. These cells first gained major attention when they were visualized intravitally using *Cx$_3$cr1^{gfp}* reporter mice, which revealed their sheer abundance, strategic positioning in the lamina propria, and special access to the gut lumen (31, 96). The generation of intestinal macrophages requires CCR2 expression, suggesting the involvement of monocyte recruitment (55, 87, 97). Indeed, when conditionally depleted with a diphtheria toxin-based approach, intestinal macrophages can be efficiently replenished by adoptive Ly6C^{hi} monocyte grafts (98, 99). We note that gut macrophages display a uniquely short half-life of 3 weeks (98, 100), compared with other tissue macrophages (38), matching the dynamic gut landscape with its constant tissue renewal. The bulk of intestinal macrophages hence rely on constant renewal by Ly6C^{hi} monocytes entering the gut tissue.

Monocytes that enter the healthy gut are exposed to locally prevalent microbial stimuli and respond to these by cytokine production. Indeed, this response likely contributes to setting the steady-state tone of the mucosal immune system that is required to prepare the organism for future insults and pathogen challenges at this site (101). More recently, IL-1β produced by intestinal CX_3CR1^+ macrophages was shown to trigger innate lymphoid cells to secrete CSF-2 and thereby prime and condition the intestinal DC compartment for its interaction with T cells (102). Low-grade homeostatic inflammation is thus required to establish the healthy and robust steady state that allows the coexistence of the host with its commensal microbiota. In further support of this notion, Trem-2-expressing intestinal macrophages promote epithelial wound repair (103). However, commensal bacterial products of the healthy gut at the same time form an environment that conditions infiltrating monocytes to avoid collateral damage by overt secretion of proinflammatory cytokines (86, 87, 104). In the colon, this homeostatic circuit involves T regulatory cells that constitutively produce the anti-inflammatory cytokine IL-10 (105), which controls hyperactivation of the CX_3CR1^+ macrophages (106). Interestingly, when monocytes enter the acutely inflamed gut, this conditioning process fails, and the cells give rise to proinflammatory effector cells that then actively promote gut inflammation (87). Monocyte fates in the lamina propria have thus emerged as a unique paradigm that might allow the definition of the molecular cues that govern the differentiation of monocyte-derived macrophages in a healthy nonpathological tissue context, as well as in a pathological context. The intestine also hosts macrophages in the muscularis layer (107). These cells were recently shown to influence the pattern of smooth muscle contractions by secreting bone morphogenetic protein 2 (BMP-2), which activates enteric neurons (108). In turn, neurons provide CSF-1, which is critical for the development of these cells (108); similar to lamina propria–resident macrophages, these cells are derived from monocytes (M. Bogunovic, personal communication).

Importantly, it has recently been shown that yolk sac– and fetal liver–derived resident intestinal lamina propria macrophages are present during the neonatal period, but they fail to persist in the adult intestine and are completely replaced at the time of weaning by BM-derived $Ly6C^{hi}$ monocytes (109). Thus, the intestine is a unique example of a tissue-specific context that imprints the absolute substitute of embryo-derived resident macrophages by BM-derived monocytes.

Healthy skin was also shown to harbor in the dermis a sizable population of monocyte-derived macrophages that coexist with embryo-derived macrophages (110, 111). Both the gut and skin are exposed to microbes and their products, which might trigger tonic low-grade inflammation in these tissues and thus provide a stimulus for constant monocyte recruitment. Interestingly, this does not apply to the liver macrophage compartment, which is also constitutively exposed to portal blood that is enriched for microbiota-derived antigens. Rather, KCs seem to self-maintain independently of monocytes, and the compartment seems to resist replacement by adult monocyte-derived cells, at least during acute inflammatory settings of drug-induced liver injury (35, 88). Moreover, despite the prominent exposure of the alveolar space to microbes and their products, lung macrophages are also not readily replaced by monocyte-derived cells (35, 40). It was recently observed that monocyte-derived infiltrates are present in the heart and progressively replace embryo-derived resident cells (39, 112). Arguably, the heart is not particularly exposed to bacterial products, and the trigger for the homeostatic replacement of heart macrophages could be the wear and tear of this mechanically stressed tissue. Finally, monocyte-derived macrophages are also a characteristic feature of tissues that undergo extensive postnatal cyclic development, such as the female mammary gland (79) and myometrium (113), highlighting the association of these remodeling processes with inflammation (3).

All things considered, it currently remains unclear why in tissues such as the gut, skin, and heart embryonic macrophages fail to compete with the monocyte infiltrates for the niches that allow

persistence and are likely of limited availability. Conversely, why do embryo-derived macrophages prevail and predominate, even after an inflammatory episode in other organs, such as the liver and brain (46, 88)? The end result might depend on the conditions under which monocytes enter the tissue (i.e., the quantity and quality of the inflammatory reaction and initial stimulus). Certain stimuli might thus drive monocytes to differentiate into cells that contribute activities to the resolution of inflammation but fail to acquire the capacity of long-term repopulation of the macrophage compartment. Alternatively, severe insults could compromise the resident macrophage population and promote its replacement. After their depletion by clodronate liposomes, embryo-derived heart macrophages are, for instance, replaced by monocyte-derived cells (39). It remains unclear whether these cells persist for a prolonged period of time. Most embryo-derived macrophages are also replaced by BM-derived cells upon whole body irradiation (41), although, as argued above, the repopulation might not be by monocytes, but rather by earlier myeloid CCR2-dependent precursors. In this setting, the repopulation potential and function of host macrophages is probably affected by irradiation. Interestingly, and highlighting the competition between donor and recipient cells, host tissue macrophages can recover, if the graft itself is hampered, for instance, by a CSF-2R deficiency (114). Collectively, monocytes can contribute to adult macrophage compartments even under homeostatic conditions. The relative contribution of monocyte-derived cells in the steady state might be an indicator of a para-inflammation state of specific tissues (3) or their exposure to microbial stimuli.

Maintenance of Tissue Macrophages in Homeostasis and Restoration Following Challenge

The long-term persistence of embryo-derived tissue macrophage compartments necessitates mechanisms that replenish exhausted or apoptotic macrophages during adult life. Replacement of these cells could occur through a tissue-resident stem cell–like population with the potential for asymmetric cell division. Evidence for the existence of such a population is, however, currently missing. Alternatively, differentiated macrophages could be inherently endowed with self-renewal potential. Indeed, early experiments indicated that tissue macrophages, such as microglia or alveolar macrophages, have the potential to proliferate in vitro when exposed to cytokines or plated on feeder cells (115). Interestingly, the experimentally induced combined deficiency of the transcription factors MafB and MafC was reported to enable functional macrophages to extensively self-renew (116). This established that terminal differentiation and proliferation of macrophages are compatible. Furthermore, it was shown that massive local expansion of tissue-resident macrophages, rather than monocyte recruitment, is a hallmark of TH2-dominated antiparasite responses (117). Differentiated tissue macrophage populations display a low steady-state proliferation rate (8). For peritoneal macrophages this proliferation was shown to be a stochastic event, in that already divided and nondivided macrophages have the same probability of entering the cell cycle (41). Local repopulation has hence been interpreted to rely on bona fide tissue macrophage proliferation rather than stem cell–like precursors (41). However, variations of this scheme might exist. Thus, fate mapping studies suggested that the adult epidermal LC network might not be formed by mature coequal LCs, but rather by adjacent proliferative units comprising dividing LCs and their terminally differentiated daughter cells (118). Importantly, proliferation not only ensures homeostatic macrophage cell numbers, but can also mediate their repopulation following extreme conditions associated with tissue macrophage depletion (119, 120). Moreover, macrophage proliferation likely also contributes to the striking rapid repopulation of embryo-derived macrophage compartments after their experimentally induced conditional ablation (121).

The self-renewal of tissue macrophages, although seemingly similar to that of stem cells, significantly differs from the latter because it results in the generation of two daughter cells, which are identical with respect to their identity and genetic imprint signature. In contrast, true stem cells undergo asymmetric division to maintain the stem cell pool and at the same time generate more differentiated progeny.

The homeostatic proliferation of macrophages involves the growth factors CSF-1 and CSF-2 (41) and is regulated by the transcription factors MafC; MafB (116); and, in the case of peritoneal macrophages, Gata-6 (122, 123). IL-4, however, seems to be the main driver of Th2 inflammation-driven pleural macrophage proliferation, which acts independently of CSF-1 (124). For further and in-depth discussion of this topic, we refer the reader to Reference 125 for a recent excellent review.

FUNCTIONAL SPECIALIZATION OF TISSUE MACROPHAGES

Generic Macrophage Functions

Macrophages are immune cells specialized in the phagocytosis and neutralization of cellular debris and potentially hazardous agents, including pathogens. As such, they can be considered an adaptation of multicellular organisms to cope with the challenge arising from progressive cell specialization and complexity by outsourcing immunosurveillance to specialized phagocytes. Tissue macrophages are nonmigratory cells that monitor their immediate, local environment, as strikingly visualized using intravital microscopy for microglia (126, 127). Moreover, many macrophage effector functions might be expected to be concentrated on their close surroundings. In this respect, macrophages critically differ from highly mobile DCs, which are also immune sentinels but are specialized in triggering remote T cell responses upon their translocation to tissue-draining lymph nodes (13).

To fulfill their role as guardians, tissue macrophages are equipped with a vast array of sensing molecules, including scavenger receptors, pattern recognition receptors [Toll-like receptors (TLRs), C-type lectin receptors, RIG-I-like receptors, Nod-like receptors], nuclear hormone and cytokine receptors, and adhesion molecules. The repertoire of these receptors, however, varies among tissue macrophages and likely reflects local adaptation.

Generic effector functions of macrophages include activities associated with their highly developed lysosomal compartment that bears critical protease and bactericidal activity (128). Moreover, macrophages play a critical role in orchestrating the inflammatory reaction by provision of chemokines and cytokines, which recruit and activate neutrophils, monocytes, and lymphocytes. Macrophage activation and polarization have been under intense study, mainly using in vitro systems (see the sidebar Macrophage Activation, Polarized Subsets, and Plasticity). In response to challenge, macrophages also produce a plethora of effector molecules, including a battery of growth factors, such as platelet-derived growth factors, insulin-like growth factors, hepatocyte growth factors, and fibroblast growth factors.

The expression of sensors renders macrophages responsive to changes in their environment but bears the inherent risk of macrophage hyperactivation and resulting collateral damage. Counterbalancing their stimulation, macrophages hence are subject to silencing programs that most likely set tissue-specific thresholds for their activation and allow the cells to gradually respond and gauge the quality and intensity of the stimulus. The exact nature of these silencing circuits differs between tissues and includes innate inherent suppression, as well as acquired deactivation triggered in response to activating stimuli (129, 130). Intestinal gut macrophages, for instance, express the IL-10 receptor and must be exposed to homeostatic T regulatory cell–derived IL-10 to prevent colitis (106). Another showcase for homeostatic mechanisms that curb tissue macrophage

MACROPHAGE ACTIVATION, POLARIZED SUBSETS, AND PLASTICITY

Tissue macrophages display remarkable plasticity that allows them to efficiently adjust to rapidly changing environmental signals and respond with distinct activities. Macrophage activation was proposed to occur in a bipolar mode, generating classically activated (M1) and alternatively activated (M2) macrophages with proinflammatory and resolving features, respectively (130, 212). Other schemes have introduced a macrophage designation based on fundamental homeostatic functions, such as host defense, wound healing, and immune regulation (213). However, considerable accumulating evidence, in particular from in vivo systems, points to an even broader functional spectrum of these cells. The conceptual framework of macrophage activation hence requires reassessment to accommodate current findings (214). Recent guidelines to describe macrophage activation have been based on three principles: the source of macrophages used, the definition of the activators, and a consensus collection of activation markers (215), referring mainly to cells cultured in in vitro systems. The application of these guidelines to macrophages studied ex vivo or in vivo will require additional detailed information on genetic background and animal husbandry, the disease model used, cell isolation procedures, time points of the macrophage isolation and analysis, and analytical methods. Importantly, murine macrophage insights will also have to be integrated with human data, such as a recently generated comprehensive data resource comprising hundreds of macrophage transcriptomes triggered by a diverse set of stimuli (216). We note that network modeling analyses of these data revealed at least nine distinct macrophage activation programs, thus considerably extending the original M1/M2 polarization scheme to a spectrum model. Interestingly, integrating with available murine data (135) led to a refined, activation-independent core signature for human and murine macrophages (216). Collectively, well-standardized resources should serve as a framework for future research into the regulation of macrophage activation.

activation involves the pathways controlling microglia quiescence. Microglia are believed to be silenced by TGF-β (131), as well as homeostatic neuronal-derived chemokine CX_3CL1, which dampens the neurotoxicity of the cells (132). In addition, microglia and alveolar macrophages are kept in their resting state through interactions of the OX-2 membrane glycoprotein CD200 and its receptor CD200R, as documented by the finding that both of these populations display signs of spontaneous activation in CD200-deficient mice (133, 134). We note that the robust silencing programs of tissue-resident macrophages might need more plastic cells to cope with acute challenges. This need seems to be fulfilled through on-demand recruitment of monocytes, as a flexible emergency squad (47) (**Figure 2**).

The immune sentinel task of tissue macrophages could arguably be performed by a stereotypic macrophage prototype, with minor adjustments to the particular tissue context. Surprisingly, depending on their tissue of residence, macrophages display highly distinct and characteristic gene expression signatures (135). Moreover, this striking tissue specification is also reflected at the epigenetic level (i.e., differential histone marks that indicate the state of active and, in particular, poised enhancers) (136). Collectively, these findings suggest that, beyond their unifying features, tissue macrophages display functions associated with homeostasis of their particular tissue of residence. This is quite compatible with the new discovery that these cells develop alongside their tissue of residence, rather than being eventually seeded.

The heterogeneity of tissue macrophages likely results from their adaptation to specific host tissue environments and reflects a functional polarization governed by local tissue-derived signals; with few exceptions, however, the identity of these signals remains unknown (**Figure 4**). It is also unclear whether functional heterogeneity is a result of irreversible lineage-specific differentiation or a consequence of the continuous, but reversible, induction of diverse functional programs by

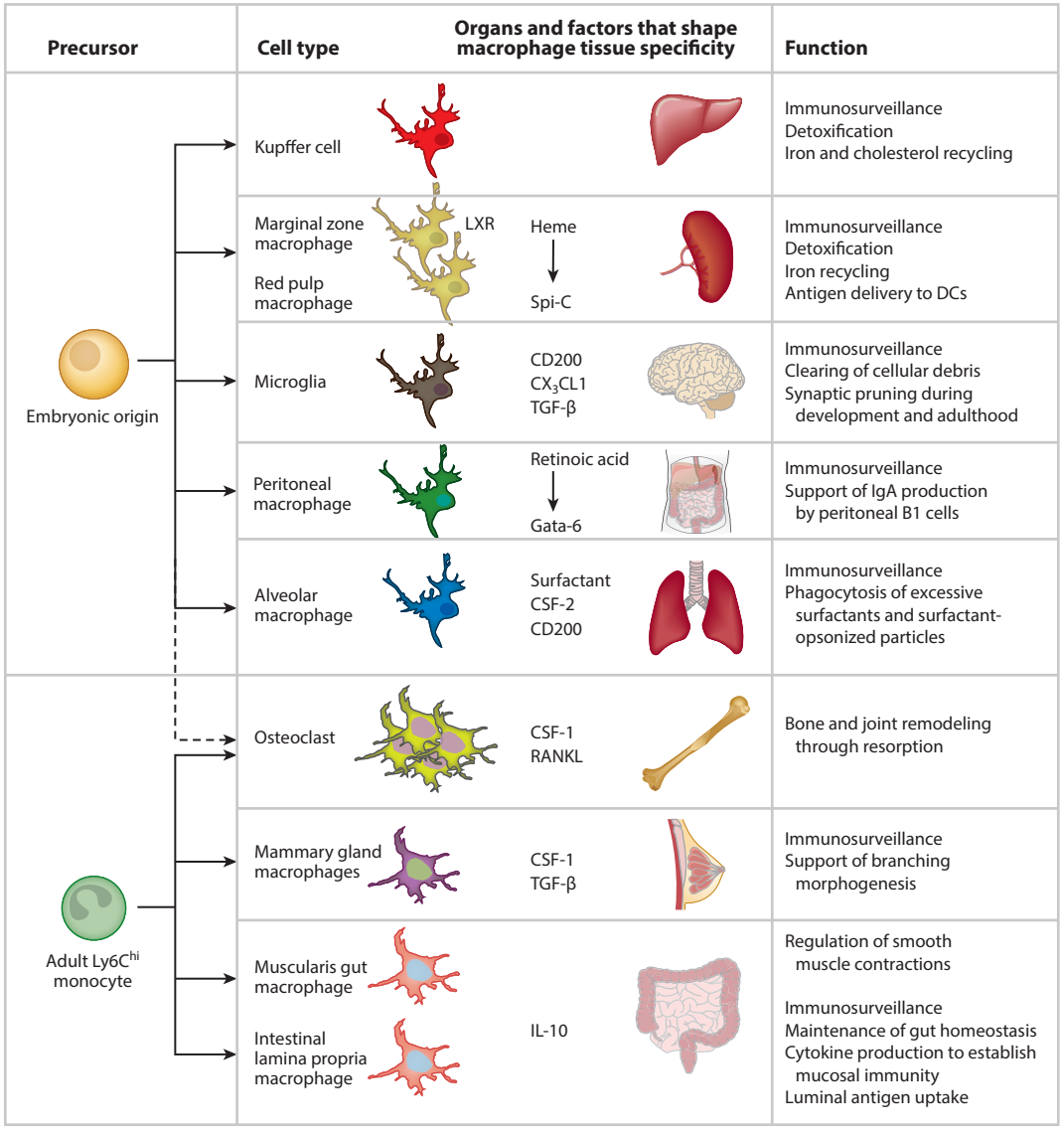

Figure 4

Factors imprinting tissue macrophage fate and maintaining specific tissue macrophage features. In their target organs, tissue-resident macrophages of either embryonic or adult monocytic origin are exposed to tissue-specific factors, which influence their development, polarization, and function. This includes the induction of an acquired deactivation state through exposure to factors such as TGF-β, CX_3CL1, IL-10, and CD200, which might prevent early, premature immune activation. Other factors, such as retinoic acid, which induces expression of Gata-6 in peritoneal macrophages, or surfactants in the lung, contribute to the proliferation, migration, and activation behavior of the cells. An additional example is heme, which was shown to induce Spi-C expression by red pulp macrophages. Abbreviation: DC, dendritic cell.

tissue-specific cues. Below we discuss selected macrophages with respect to their tissue-specific contributions. Wherever possible, we review recent progress in our understanding of the local instructing signals that govern tissue specification.

TISSUE- AND ORGAN-SPECIFIC CONTRIBUTIONS OF MACROPHAGES

The Lung

The lung is an essential respiratory organ and is constantly exposed to microbes, pollutants, and dusts. A prime task of the lung is thus to tolerate inhaled innocuous stimuli, as well as the recently appreciated lung microbiome (137), while preserving the capability of mounting an immune response against opportunistic pathogens. Dysregulation of the response can lead to infections and sustained susceptibility to allergic airway inflammation. This challenge resembles that of the intestinal tract, and indeed, both organs host equivalent innate immune cells in the lamina propria, such as monocyte-derived macrophages and $CD103^+$ and $CD11b^+$ DC subsets (138). In the lung, these cells are directly involved in acute immune responses, as well as tolerance induction (139). However, in contrast to the intestine, where constant turnover and differentiation of $Ly6C^{hi}$ monocytes into tissue macrophages ensure organ functionality (see above), in the steady state alveolar macrophages rely on cells characterized by fetal-monocyte origin, CSF-2 dependence, and longevity (40). Importantly, these macrophages are located not in the interstitial space, but in the alveoli of healthy airways. Whether the reliance on embryonic origin applies to all lung macrophages remains to be determined.

Classical airway alveolar macrophages interact with alveolar epithelial cells through CD200 and TGF-β signaling, which ensures their noninflammatory state (134, 140). Additionally, on their luminal surface, type II alveolar epithelial cells express pulmonary surfactant-associated proteins, which bind to immune receptors, such as SIRPα, TLR2, TLR4, and their coreceptors. Moreover, specific surfactant-associated proteins can bind pathogens, apoptotic cells, and allergens to facilitate their neutralization and directly inhibit proinflammatory gene activation (141). As a consequence, alveolar macrophages isolated from mice deficient in surfactant-associated protein D show spontaneous expression of metalloproteinases, as well as oxidant production, resulting in chronic inflammatory alterations (142). Yet alveolar macrophages are important to clear excessive surfactants by phagocytosis. This activity is at least in part controlled by the transcription factor Bach2, whose absence leads to the accumulation of lipids and subsequent development of a pulmonary alveolar proteinosis (PAP)-like disease (143).

The respiratory epithelium is the major source of CSF-2, which is necessary for the differentiation of fetal monocytes into alveolar macrophages (40). Accordingly, CSF-2-deficient mice are characterized by reduced numbers of alveolar macrophages (40). Moreover, the remaining cells are functionally impaired, resulting in lipid accumulation and PAP manifestation. Surfactants and high CSF-2 levels are potential local factors that imprint the unique phenotype and functions of alveolar macrophages. Indeed, alveolar macrophages features can be partially conferred to macrophages isolated from the peritoneal cavity upon their transfer into the lung (136, 144).

Alveolar macrophages also excel in bacterial phagocytosis and produce significant amounts of type I interferon upon pulmonary virus infection (145). They thus contribute to the development of inflammation but may also be involved in its resolution, as alveolar macrophage depletion before bacterial or viral infection results not only in decreased pathogen clearance, but also in exacerbated inflammation (146, 147). This restorative activity of lung alveolar macrophages can potentially be explained by their robust anti-inflammatory phenotype, which needs to be overcome by the

pathological stimuli. Indeed, a higher homeostatic activation status of alveolar macrophages by genetic deletion of factors such as A20 and CD200R results in improved resistance to infection (134, 148).

In conclusion, the unique microenvironment of the lung, as a classical environmental barrier tissue, significantly contributes to the phenotype of alveolar macrophages by providing factors that control hyperactivation and thereby govern the characteristic dampened tolerogenic immunological response in this tissue. For a detailed review on alveolar macrophages, we refer the reader to Reference 149.

The Liver: Kupffer Cells

The liver is a vital organ in charge of efficient uptake of nutrients, amino acids, carbohydrates, lipids, and vitamins and their subsequent storage. It further promotes metabolic conversion and release of metabolites into the blood and bile, as well as the detoxification of ingested hazardous chemicals. Liver lobules receive continuous blood supply from the arterial circulation and the portal vein, which drains the gastrointestinal tract. This anatomical setting results in prominent liver exposure to food antigens, microbial products, and xenobiotics. The liver hence stars prominently in host defense and the establishment of local and systemic tolerance. Blood entering the hepatic parenchyma passes through a network of thin-walled sinusoids, which contain specialized nonparenchymal cells, including a fenestrated layer of liver sinusoidal endothelial cells. These features confer effective antigen capture and presentation in this microenvironment (150).

KCs, the resident macrophage population of the liver, are most abundant in the upstream periportal regions of the liver sinusoids. They display high phagocytic and lysosomal activity, which highlights their specialization in the surveillance and filtering of blood entering the sinusoids. They thereby form a protective barrier preventing systemic pathogen circulation. In addition, KCs remove potentially harmful endogenous compounds, such as complement and fibronectin-coated particles, material released from dying cells, and extracellular matrix components, as well as immune complexes to avoid intravascular coagulation. Despite, and perhaps even because of, their continuous exposure to gut-derived antigens and bacterial endotoxins, KCs exhibit a tolerogenic phenotype in the healthy liver. However, under disease conditions, they can shift to a pathologically activated state and then cause hepatocellular injury and damage (151, 152).

KCs also perform liver-specific metabolic functions. The liver is a major site of iron recycling from senescent erythrocytes. Using distinct scavenger, Fc, and complement receptors, KCs efficiently clear these malformed or oxidatively damaged red blood cells from the circulation. Moreover, KCs remove hemoglobin-containing vesicles released during the life span of erythrocytes (153). Their expression of the scavenger receptor CD163 allows endocytosis of hemoglobin–haptoglobin complexes, thereby protecting tissues from free hemoglobin-mediated oxidant injury (154). KCs furthermore regulate plasma cholesterol levels by removing native and modified lipoproteins from the circulation. Specifically, they express receptors for both high-density lipoproteins and low-density lipoproteins (LDLs) but are primarily involved with the catabolism of LDLs and modified LDLs (152).

Bone: Osteoclasts

The bone is a unique, highly vascularized, rigid, yet dynamic organ designed to provide maximal strength with minimal mass. Bone shape is dictated by constant remodeling involving bone synthesis by mesenchymal osteoblasts and bone resorption by specialized bone-resident macrophages, the osteoclasts. Abnormalities in the balance of osteoclast development or function, relative to

osteoblast activity, result in skeletal pathologies, such as osteopetrosis or osteoporosis (155). Moreover, osteoclasts are essential for normal long bone formation during embryogenesis (156). They are multinucleated cells, or polykaryons, that form by cell fusion near the bone surface. Mice that harbor mutations in the genes encoding CSF-1 (157) or receptor activator of nuclear factor kappa-B ligand (RANKL) (158) develop osteopetrosis, establishing that these factors are critical for in vivo osteoclastogenesis. Additional significant roles in osteoclast differentiation and commitment are performed by the transcription factors PU.1 (159) and c-Fos (160). RANKL is expressed by osteoblasts and induces the polarization of mature osteoclasts, which is manifested in structural changes essential for their bone resorption. This includes cytoskeletal rearrangements that enable the formation of a ruffled membrane structure and a sealing actin ring toward the bone surface (161). Osteoclast polarization is also controlled by integrin $\alpha v\beta 3$-mediated adhesion and signaling (162), the tyrosine kinase c-Src (163), and microRNA circuits (164). Following polarization, the resorption organelle is acidified by the osteoclast-specific proton pump ATP6i followed by the release of lytic enzymes into the resorption pit (165). Both the differentiation and function of osteoclasts are inhibited by osteoprotegerin (OPG), a soluble decoy receptor for RANKL, which is also produced by osteoblasts (166). The generation of osteoclasts is thus dictated by the balance of RANKL and OPG by osteoblasts.

Pre- and postnatal osteoclast ontogeny remains poorly defined. Specifically, it is unclear whether osteoclasts originate from circulating blood monocytes or from bone tissue–resident precursors. Ly6C^{hi} monocytes can efficiently migrate from the circulation back to the BM (59) and could thereby contribute to ostoclastogenesis, in particular in marrow-less bones. Interestingly, CCR2-deficient mice suffer from high bone mass owing to a decrease in the number, size, and function of osteoclasts (167). It was suggested that CCR2 activation induces the RANK surface expression in osteoclast progenitor cells. Yet impaired remodeling could also result from the reduced BM egress of Ly6C^{hi} monocytes in *Ccr2*$^{-/-}$ mice (54). The Ly6C^{hi} monocyte subset was also shown to be critical for osteoclast-mediated bone destruction in inflammatory arthritis mediated by the expression of Fcγ receptor IV (168). Finally, a recent photoconversion-based cell tracking and parabiosis study established that in the steady state, osteoclasts can be generated from circulating monocytes (169). The stimulus that recruits the monocytes to the bones, however, remains to be defined.

The Brain: Microglia

Microglia represent the tissue-resident macrophages of the CNS and the sole resident hematopoietic, mesodermal-derived cell type in the CNS parenchyma. Microglia fulfill two main functions in the adult CNS: homeostatic maintenance and immune defense. Besides the neuronal circuitry, the CNS comprises additional macroglia populations, such as the myelin sheath-forming oligodendrocytes and astrocytes, which are involved in physiological and biochemical tasks to functionally support the BBB. The BBB critically contributes to the immune-privileged state of the CNS and secures the restricted passage of substances from the bloodstream into the CNS.

Aside from epidermal LCs (170), microglia were the first cells of the innate immune system that were experimentally proven to be long-lived and independent of BM-derived myeloid cell replenishment during adulthood (171, 172). However, compared to LCs and other peripheral tissue-resident macrophages, murine microglia exclusively derive from the embryonic yolk sac, without a contribution from the fetal liver (26, 37). This might be due in part to the presence of the BBB, which might limit the CNS entry of myeloid cells in the adult. Alternatively, embryonic yolk sac macrophages might be uniquely able to infiltrate the neuroectoderm, owing to their expression repertoire of matrix metalloproteinases or chemokine receptors (43).

In the developing embryonic CNS, microglia undergo a rapid proliferation and increase in cell numbers, but steady-state proliferation largely ceases in adulthood (37). Interestingly, when adult microglia are acutely ablated using either a diphtheria toxin-based approach (121; A. Waisman, personal communication) or a CSF-1 receptor kinase inhibitor (173), this cellular compartment displays a remarkable potential to restore itself. If the ablation is accompanied by BBB breakage, infiltrating BM-derived cells also can contribute to replenishing the brain macrophage compartment (174; A. Waisman, personal communication). The existence of these mechanisms suggests that a microglia-free brain parenchyma is incompatible with brain function and viability and thus supports the emerging concept that microglia are critical for CNS homeostasis.

Microglia are indeed tightly imbedded into the cellular communication with neuroglial cells. Early infiltrating embryonic microglia are highly phagocytic and are probably involved in the clearance of cellular debris (43). Microglia actively engulf synaptic material and perform synaptic pruning during postnatal development (22, 175, 176). Disruption of this process, for instance, through a CX_3CR1 deficiency, leads to impaired functional brain connectivity and disturbed social behavior in adult mice (177, 178). Additionally, during adulthood, microglia seem to be involved in the clearance of neuroprogenitors in the subgranular zone of the hippocampus through apoptosis-coupled phagocytosis (179). The prominent expression of purinergic receptors of the P2Y family on microglia (131) renders these cells sensitive to adenosine triphosphate and its metabolites (e.g., alert factors released into the extracellular space after neuroglia injury) (126). Interestingly, microglia have also been proposed to secrete brain-derived neurotrophic factor, critically supporting the formation of learning-induced spines in the motor cortex (121). These examples demonstrate that microglia actively influence and shape the development of the synaptic network, but microglial-neuronal communication is not unidirectional. Thus, neurons express IL-34, the alternative ligand for CSF-1R, and the absence of this factor results in a severe reduction of microglia in all parts of the cerebrum, comparable to that of CSF-1-deficient mice (180, 181). This indicates a critical ongoing dependence of microglia on functional CSF-1R signaling, as also supported by recent results from studies involving CSF-IR blockade (173). An additional role of neuronal-microglia cross talk is associated with the maintenance of microglia quiescence. Neuronal CX_3CL1, CD200, and exosome-derived miR-124 were shown to control microglial cell-autonomous neurotoxicity in various CNS inflammation models (132, 133, 182). Neuronal or oligodendroglia-derived TGF-β contributes to their inactivated phenotype and establishes a unique genetic microglia signature (131, 183). Interestingly, upon BM transplantation, HSC-derived cells presumably can gain longevity and even radioresistance, two hallmarks of microglia (A. Volasky, S. Jung, unpublished observation). How the CNS parenchyma imprints these features, however, needs to be investigated. In summary, recent advances in our understanding of microglial biology indicate that these cells represent an important component of the CNS, which is imprinted by its neuroglial surrounding and linked to its unique secluded location behind the BBB.

Splenic Macrophage Populations

The spleen is the largest lymphoid organ in the body and the main site at which T and B cell responses to blood-borne antigens are initiated. Because this organ lacks afferent lymphatics, functions of the spleen are centered on the systemic circulation. The spleen serves as a filter of the blood for senescent cells, including erythrocytes, and potential noxious materials. Many of the above functions are essentially attributed to specific macrophage subpopulations that are compartmentalized into different splenic domains and are well adapted to perform site-specific homeostatic roles (184).

Red pulp macrophages (RPMs) are specialized in iron recovery and prominently express proteins involved in all recycling phases, starting from the scavenging of senescent or damaged erythrocytes, the uptake of hemoglobin, and finally heme breakdown and iron export (185). Similar to KCs, RPMs also use CD163 for endocytosis of hemoglobin–haptoglobin complexes released from dying erythrocytes (154). The development of murine RPMs, which are characterized as F4/80$^+$ CD206$^+$ CD11b$^{lo/-}$ cells, requires the transcription factor Spi-C; accordingly, Spi-C-deficient mice exhibit an impaired clearance of erythrocytes and develop a selective iron overload in the splenic red pulp (186). In an intriguing twist, heme was recently shown to induce Spi-C by releasing its gene from control by the transcriptional repressor Bach 2 (187). This provides the first evidence for metabolite-driven differentiation of a specific tissue-resident macrophage. RPMs are generated prenatally and maintained through steady-state adulthood without substantial monocytic input (35, 41). Yet, in the case of iron overload toxicity, which acutely eliminates RPMs, heme moieties can trigger Spi-C expression in monocytes, and these cells might contribute to the reestablishment of the RPM compartment (187). The splenic red pulp is also a site for the storage and rapid deployment of monocytes, a potential resource that the body can exploit to regulate inflammation (64).

The interface of the splenic white and red pulp, called the marginal zone, is a reticular network and hosts two distinct resident macrophage populations that are strategically positioned to screen entering arterial blood: the marginal zone macrophages (MZMs) and the marginal metallophilic macrophages (MMMs). As a result of the splenic anatomy, blood flow slows down in the marginal zone, and pathogens present in the systemic circulation are efficiently removed by the macrophage subsets (188, 189). These macrophages also regulate the clearance of apoptotic cells and may be involved in the delivery of cell-associated antigens to CD8$^+$ splenic DCs (190). The differentiation of both MMM subsets requires the nuclear receptor LXRα, and LXR-deficient mice are defective in mounting an immune response to blood-borne pathogens (191, 192). Interestingly, the adoptive transfer of wild-type monocytes into LXR-deficient mice leads to the functional reconstitution of splenic MMM subsets (192), although it is unclear whether these cells persist. The elimination of MZMs and MMMs leads to the accumulation of apoptotic cells in the white pulp and their uptake by RPMs, resulting in increased inflammatory cytokine production, enhanced adaptive immunity, and accelerated autoimmunity in disease-prone mice (193). This suggests that MMMs and MZMs are, as opposed to RPMs, specialized in the quiescent removal of their cargo.

As for the distinct roles of the two cell populations, MZMs are positioned within the outer layer of the marginal zone and express a unique set of pattern recognition receptors, such as CD204, macrophage receptor with collagenous structure (MARCO), and the C-type lectin CD209b (SIGN-R1), which contribute to the capture of blood-borne antigens (188, 194, 195). MZMs intimately interact with a specialized noncirculating type of mature B cells that segregates anatomically into the marginal zone. These IgM^{hi} B cells are reactive to bacterial cell wall components, display a lower activation threshold than do follicular B cells, and are critical for the integrity of the marginal zone architecture (196). Marginal zone B cells secrete chemokines, such as CCL19 and CCL21, that are important for the recruitment and retention of MZMs (197) and regulate SIGN-RI expression on MZMs (198). In turn, the expression of MARCO by MZMs has been implicated in the retention of these B cells (199), and IgM responses to polysaccharides depend on B cell colocalization with MZMs (200).

MMMs are located adjacent to the white pulp and marginal sinus and form an inner ring of macrophages. They are characterized by the expression of CD169 (SIGLEC1-sialic acid–binding immunoglobulin-like lectin 1), which mediates, for instance, the engulfment of sialic acid–expressing bacteria (201). MMMs surround the B cell follicles, and their presence depends on follicular B cells (196). As such, these cells might be functionally similar to their lymph node

counterparts, the CD169^{+} subcapsular sinus macrophages, which have been shown to transfer particulate antigens to B cells for deposition on follicular DCs (202, 203). Following antibody-mediated targeting of antigens to CD169^{+} MMMs, these cells can also transfer antigens to CD8^{+} DCs for cross presentation and the subsequent activation of a cytotoxic T cell response (204). Moreover, MMMs are the main producers of type I interferon after a viral challenge and are thus believed to contribute to antiviral responses (205).

Macrophages are also located in the white pulp, which is the site where immune responses to blood-borne antigens are initiated and which structurally resembles lymph nodes (188). White pulp macrophages can be identified by their common expression of the pan macrophage marker CD68. They include tingible body macrophages, which are located in the germinal center (GC) of B cell follicles and are responsible for the clearance of phosphatidyl-serine-expressing apoptotic B cells that arise during the GC reaction. To this end, these macrophages are equipped with specific receptors, such as the Milk fat globule-EGF factor 8 (MFG-E8). MFG-E8 deficiency results in the failure to remove apoptotic B cells and leads to autoimmunity (206).

Collectively, and in line with the complex architecture and multiple tasks of their organ of residence, splenic macrophage subsets display considerable phenotypic and functional heterogeneity. It remains unresolved whether these macrophage subsets are different cellular entities with independent ontogeny or share precursors that are differentially educated in the distinct splenic microanatomical niches. Thus, the spleen might represent an excellent model to study site-specific regulatory networks of development and/or function.

Peritoneal Cavity Macrophages

Macrophages isolated from the peritoneal cavity of mice are the best-studied resident cell population among the macrophage families and therefore have contributed significantly to our current understanding of macrophage biology. Isolated peritoneal macrophages serve as a readily accessible macrophage source to study signaling cascades, phagocytosis, and cytokine and chemokine secretion after pathogen stimulation. Despite decades of intensive research, the tissue-specific function and ontogeny of peritoneal macrophages remain largely unknown. Peritoneal macrophages are heterogeneous and consist of two subsets, which differ phenotypically and functionally (207). A minor fraction of F4/80^{int} CD11b^{int} peritoneal macrophages was proposed to represent an intermediate, on-demand precursor for the F4/80^{hi} CD11b^{hi} peritoneal macrophage population (208). Alternatively, larger F4/80^{hi} CD11b^{hi} cells were proposed to represent mature peritoneal macrophages, which dominate in the peritoneal cavity under steady-state conditions. These cells are of prenatal origin and display longevity and limited self-renewal (35). Under inflammatory conditions, this population vanishes during the first days of the response, which is known as the macrophage disappearance reaction (119), but reappears thereafter, possibly via extensive proliferation (120). The early fate of peritoneal macrophages under these inflammatory circumstances still remains a matter of debate, and explanations, which are possibly not mutually exclusive, include cell adhesion to the serosal mucosa, their draining to lymph nodes or omenta, and cell death. Ly6C^{hi} monocytes, which infiltrate the inflamed peritoneum, can differentiate during the recovery phase of this ablative condition, first into small F4/80^{int} CD11b^{int} macrophages (207, 209) and subsequently into large F4/80^{hi} CD11b^{hi} cells, thus seemingly integrating into the peritoneal macrophage network (35). It remains, however, to be shown if these cells also become true functional equivalents of embryo-derived peritoneal macrophages.

The maintenance of F4/80^{hi} peritoneal macrophages depends on the transcription factors Cebpβ (208) and Gata-6 (122, 123, 209). Gata-6 is specifically expressed in peritoneal macrophages

compared to other tissue-resident macrophage populations (135, 136) and seems to be involved in the tissue-specific location, function, and molecular identity of these cells. Gata-6 is induced by retinoic acid, a biologically active vitamin A metabolite that is abundantly produced by peritoneum-associated adipose tissue, the so-called omentum. The localization of peritoneal macrophages to this microanatomical site was proposed to be critical to reversibly induce Gata-6 and its associated downstream transcriptional program (209). Among other genes, Gata-6 drives TGF-β expression, which has been linked to the requirement of peritoneal macrophages for the production of IgA by B1 cells in the intestinal lamina propria (209). Gata-6-deficient macrophages also display a perturbation of metabolic regulators, including acetyl-CoA generation, which might be associated with their reduced survival (123). Interestingly, a peritoneal macrophage-like gene program can be induced in BM-derived macrophages upon enforced Gata-6 expression, which was accompanied by a phenotypical switch and retention of the cells in the peritoneum (122). Moreover, BM transfer can establish a Gata-6-expressing macrophage population in the peritoneal cavity of lethally irradiated mice (209). This suggests that a Gata-6-driven program can be initiated by both embryo-derived and adult BM-derived cells. However, monocytes that enter the peritoneum under conditions of sterile or septic inflammation might not have access to the unique instructing omentum niche. Although some of these cells enter the long-lived peritoneal macrophage pool (35), it remains to be shown whether these cells are imprinted and functionally equivalent to peritoneal macrophages.

The interaction between tissue mesothelial cells of the omentum and peritoneal macrophages illustrates another highly orchestrated example of macrophage education by local tissue-derived factors. Interestingly, the specialization of peritoneal macrophages sets a unique precedent for a thus-far unique case, in which a given tissue macrophage receives its imprint in a dedicated niche, which is distinct from the site of its subsequent residence and action. Whether such a scenario also applies to other tissue macrophages remains to be investigated.

CONCLUSION

Macrophages are central players in innate and adaptive defense reactions, directly neutralizing pathogens by phagocytosis and orchestrating responses of other immune cells, as well as stroma. However, beyond this generic role as guardians, macrophages can also be regarded as integral components of their respective host tissues. Accordingly, in response to local adaptation, tissue macrophages display significant functional specialization, which is reflected in their phenotype, as well as actual and potential gene expression profiles. Given their central role in homeostasis, inflammation, and immunity, macrophages are prime targets for therapeutic intervention. However, before their potential can be fully realized, many key questions remain to be answered. We have only begun to understand the tissue-specific contributions of macrophages, as they have arguably to be studied in the context of the intact organism. With few exceptions, we also have an incomplete understanding of the local cues that establish the distinct macrophage identities, as well as the mechanisms that maintain them. Related to this issue are questions pertaining to the interplay of embryo-derived macrophages and monocyte-derived cells that are recruited during adulthood. What are the mechanisms controlling the longevity and self-renewal of tissue macrophages? Do tissue macrophage compartments age, and can traumatic experiences early in life have long-lasting impacts on them? Do monocyte-derived macrophages that persist in tissue merely rejuvenate the prenatally established tissue macrophage compartments? Or do they add novel functions and features? Does the distinct ontogeny of the cells dictate their activities, or are the cells plastic and can truly converge on a functional equivalent? Finally, we have to expand our knowledge to the human body and its specific disease conditions.

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