Masters of Immunology

Macrophages: Gatekeepers of Tissue Integrity

Yonit Lavin and Miriam Merad

Abstract

Macrophages form a heterogeneous group of hematopoietic cells that reside in tissues, where they are required to maintain organ integrity. Tissue macrophages contribute to tissue formation, metabolism, homeostasis, and repair. They have a unique ability to sense and respond to tissue damage. They serve as the first line of defense during infection and help promote immune tolerance in the steady state. Although most tissue macrophages share a high phagocytic and degradative potential, they are heterogeneous in origin, as well as in homeostatic function and response to insults. Here, we will discuss recent developments in our understanding of the origin of tissue macrophages and their functional specialization in tissues. Cancer Immunol Res; 1(4); 201–9. ©2013 AACR.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should acquire a basic knowledge of the heterogeneity of macrophages, their roles in homeostasis, infection, and in the maintenance of organ integrity. A better understanding of the cellular ontogeny and tissue regulation of this group of circulating and tissue-resident hematopoietic cells of myeloid origin, how they balance between promoting immune tolerance during steady state, and responding to tissue damage and inflammation during infection will lead to the development of more specific and effective therapeutics.

Acknowledgment of Financial or Other Support

This activity does not receive commercial support.

Introduction

Macrophages form a heterogeneous group of tissue-resident hematopoietic cells of myeloid origin that share a superior phagocytic potential and an ability to recognize and respond to tissue damage and infection. Macrophages populate all tissues of the body and play a key role in the maintenance of tissue integrity and repair (1, 2). Global transcriptome analysis of purified tissue macrophage populations by the Immunological Genome Project has contributed to a better understanding of the macrophage lineage and has revealed considerable transcriptional diversity between macrophages from different organs (3), emphasizing their specialized role in different tissues. The development of engineered mouse models to trace myeloid progenitors, quantify macrophage repopulation in situ and probe macrophage regulation in vivo has also revealed novel developmental and regulatory control of the macrophage lineage. In this review, we will discuss our current understanding on the regulation of macrophage development and function that has emerged from these studies.

Macrophage Phenotype

Macrophages are characterized by specific phenotypic features and by the expression of particular markers, none of which is entirely restricted to the cell type. In mice, macrophages express the hematopoietic lineage marker CD45 and lack lineage markers of other immune cells (including Gr-1, CD3, and CD20). They express the receptor for macrophage colony-stimulating factor (M-CSF, renamed Csf1), the integrin CD11b, Fcγ receptor 1 (FcγRI) CD64, and the receptor tyrosine kinase MerTK. The latter two markers are particularly indicative of the unique function of macrophages as scavengers of foreign antigens and apoptotic cells. In certain tissues, particularly in locations where antigen presentation is critical, such as the microbial coated intestine, macrophages constitutively express cell surface MHC class II and the integrin CD11c, two molecules shared with dendritic cells (4). In humans, macrophage markers include the Csf1 receptor CD115, the FcγRI CD64, the FcγRIII CD16, and the scavenger receptors CD68 and CD163. In both mice and humans, these markers need to be used in combination to define macrophages, as none is cell type specific (see Fig. 1).
Macrophage Ontogeny during Steady State and Inflamed State

The mononuclear phagocyte system

In 1908, Elie Metchnikoff (1845–1916) was awarded the Nobel Prize in Physiology or Medicine for the discovery of phagocytosis (5). Phagocytosis, derived from the Greek word phago meaning ‘to devour,’ refers to the process of engulfment of large particles by phagocytes. In mice and humans, phagocytes include mononuclear phagocytes and neutrophils, which are also called polymorphonuclear phagocytes due to the segmented shape of their nuclei. Mononuclear phagocytes include blood-circulating monocytes, tissue-resident macrophages, and dendritic cells. A foundational dogma in immunology suggests that monocytes and macrophages are part of a continuum that form the mononuclear phagocyte system, in which blood monocytes are the circulating link between bone marrow–derived myeloid precursors and tissues macrophages (6).

Recent studies have revealed that commitment to the mononuclear phagocyte lineage is determined at the stage of the macrophage–dendritic cell progenitor (MDP), at which point, erythroid, megakaryocyte, lymphoid, and granulocyte fates have been precluded (7, 8). MDP cells, phenotypically defined as lineage–c-kit–CX3CR1+CD115+, give rise to common dendritic cell progenitors (CDP), which differentiate into circulating pre-dendritic cell precursors that leave the bone marrow to repopulate the short-lived tissue dendritic cell pool (9–11). In parallel, MDP differentiate through the recently described common monocyte precursor (cMoP; ref. 12) into two subsets of monocytes that are distinguished on the basis of the expression of the lymphoid 6c (Ly6C) antigens. Ly6Chi monocytes are short-lived cells that extravasate in inflamed tissues in response to injury signals to differentiate into inflammatory dendritic cells and macrophages. Ly6Clo monocytes have distinct homing and functional properties compared with Ly6Chi monocytes (13). In contrast to Ly6Chi monocytes, Ly6Clo monocytes cannot infiltrate tissues; they patrol the endothelium and contribute to the maintenance of endothelial cell integrity (14). Recent data revealed that circulating Ly6Clo monocytes in fact likely arise from Ly6Chi monocytes, and these cells form a steady state continuum [see Fig. 2 (13)].

Tissue-resident macrophages

All tissues have resident macrophages that perform local homeostatic functions in the steady state (16). In vivo radioisotope-labeling studies in mice exposed to inflammatory injuries and radiation chimera experiments led to the dogma that circulating monocytes are constantly maintaining the tissue macrophage pool (17, 18). However, many observations conflict with the supposed monocyte origin of tissue-resident macrophages in the steady state. For example, the first macrophages, called primitive macrophages, appear embryonically before the development of monocytes (19). In addition,
monocytopenic animals have normal tissue macrophage density (20, 21). Recently, novel fate-mapping models in the mouse have revealed that, unlike most hematopoietic cells that are constantly replenished from the bone marrow, macrophages are unique in that they are embedded in the tissue during embryonic life.

In the embryos, hematopoiesis begins in an extra-embryonic structure called the yolk sac around embryonic age E7.5. Yolk sac hematopoiesis wanes with time and is slowly replaced by new waves of hematopoiesis initiated in the embryo proper, first in the aorta–gonads–mesonephros region and later in the fetal liver (22–24). Hematopoiesis initiated in the embryo proper is called definitive hematopoiesis as opposed to yolk sac–derived primitive hematopoiesis. Fate-mapping analysis of primitive and definitive hematopoietic precursors in the embryo (25) revealed that yolk sac macrophages seed most tissue rudiment in the embryos but differentially contribute to the adult macrophage pool (F. Ginhoux and M. Merad; unpublished data). Primitive macrophages that arise in the yolk sac can maintain the homeostasis of adult brain macrophages, also called microglia, with minimal contribution from hematopoiesis that arises after E7.5 (26). Langerhans cells, the macrophage-like dendritic cells of the epidermis, are seeded initially from yolk sac macrophages. However, a second wave of fetal liver monocytes infiltrate the skin rudiment around E14.5 and almost entirely replace the yolk sac–derived macrophages for the maintenance of epidermal Langerhans cells in the adult (27). A small fraction of macrophages that derive from yolk sac precursors have also been found in several tissues of the adult mouse (28). Other tissue macrophages may be replenished by subsequent waves from the fetal liver monocytes as well, although this has yet to be shown in a fate-mapping model. Nonetheless, most macrophages in the spleen, peritoneum, liver, and lung seem to be maintained by embryonic precursors that take residence in tissues before birth, independently of adult hematopoiesis (15, 28, 29).

In humans, numerous studies have reported that tissue macrophages can still form despite strongly reduced levels of circulating monocytes (30–33). For example, in four patients with a syndrome of monocytopenia and deficiencies in dendritic cells, B lymphocytes, and natural killer cells, cutaneous macrophages were found unaffected (30). Similarly, a patient with a null mutation of the IFN response factor 8 (irf8) gene was found to have a block in monocyte and dendritic cell differentiation, but despite profound peripheral monocytopenia, macrophages were present in the lymph nodes and in the bone marrow.
The normalcy of macrophages in these cytopenic patients further suggests that tissue macrophages can develop in the absence of monocytes in humans.

Some populations of tissue-resident macrophages do arise from circulating bone marrow precursors. Mouse intestinal macrophages are replenished by bone marrow–derived Ly6C<sup>hi</sup> monocytes (34, 35). The kidney and uterine macrophages may also in part arise from bone marrow precursors (2, 28). The marginal zone macrophages located at the interface between splenic red and white pulp, where most arterial blood enters the spleen, also derive from circulating monocytes in response to liver X receptor (LXR) nuclear receptor signaling (36). It is unclear why some macrophages need to be maintained by circulating precursors and some do not, and whether inflammatory cues expressed in these specific sites contribute to their steady state turnover. These data emphasize the heterogeneity of the macrophage lineage and the limitations of the current classification of the mononuclear phagocyte system. A new conceptualization of the mononuclear phagocytes should include the diverse origins of tissue macrophages.

**Maintenance of tissue-resident macrophages in the steady state**

Local maintenance of tissue-resident macrophages is likely due to their prolonged half-life in tissues and their ability to repopulate locally. Local proliferation and survival of tissue-resident macrophages are dependent on growth factors that differ between tissues. Most tissue macrophages are dependent on Csf1 (37). However, some tissue macrophage populations remain unaffected in the Csf1op/op mouse that carries a natural null mutation of the Csf1 gene (37). Epidermal Langerhans cells and microglia among others persist in the Csf1op/op mouse and yet these cells are dependent on the receptor for Csf1 (26, 38). An alternate ligand for the Csf1 receptor, called interleukin (IL)-34, is expressed locally in the skin and brain tissues and was found recently to be critical for maintenance of these cells (39, 40). In addition to Csf1 and IL-34, granulocyte macrophage colony-stimulating factor (GM-CSF; recently renamed Csf2) is another cytokine that controls local macrophage maintenance playing an essential role in the repopulation of alveolar macrophages in the steady state (29). The cellular steps that control local macrophage repopulation remain unclear although recent studies from our laboratories revealed that tissue macrophage proliferation is not restricted to a specific compartment, suggesting that macrophages should be able to proliferate locally without relying on a dedicated progenitor population (29).

**Inflammatory macrophages**

In contrast with resident macrophages, infiltrating or inflammatory macrophages are found only in injured tissues, and they derive from circulating monocytes. Most tissue injuries lead to the recruitment of large numbers of monocytes and induce their local differentiation into inflammatory macrophages. Circulating monocytes provide a source of infiltrating macrophages in many pathologic settings, such as cancer (41), atherosclerosis (42), and metabolic disease (43). The interplay of bone marrow–derived inflammatory macrophages and tissue-resident macrophages has only recently started to be explored, and it seems to vary with the nature of the injury. For example, parasitic infection leads to IL-4–dependent expansion of tissue-resident macrophages (44), and influenza viral infection (29) leads not only to the depletion and subsequent repopulation of tissue-resident macrophages but also to the recruitment of infiltrating macrophages. Ionized irradiation, on the other hand, ablates tissue-resident macrophages and promotes their replacement by adult bone marrow–derived macrophages. However, even in this case, tissue-resident macrophages can be triggered to repopulate locally if adult bone marrow–derived precursors are impaired in their ability to differentiate into macrophages. X-ray irradiation does not definitively eliminate the tissue-resident macrophage pool but likely delays its repopulation potential, providing a competitive advantage to adult bone marrow–derived macrophages (29). Detailed ontogeny studies have not yet been carried out in chronic inflammatory conditions, but it is likely that a mixture of tissue-resident and monocyte-derived macrophages accumulate in chronically inflamed tissues such as in solid tumors (45). The exact contribution of tissue-resident and -infiltrating macrophages to inflammation and repair remains to be examined. Another important question will be to examine whether infiltrating macrophages can engraft in tissues and acquire resident macrophage function or whether similar to inflammatory dendritic cells they disappear once the inflammation resolves (4).

**Macrophages in Homeostasis and Disease**

Macrophages play a key role in organ integrity through their contribution to tissue development, homeostasis, and repair. Macrophages also take on specialized roles integral to the organ in which they reside, and their unique functions are reflected in the diversity of the tissue macrophage transcriptome.

**Homeostasis**

Tissue-resident macrophages play a key role in tissue formation and remodeling during development. Macrophages are especially important in ducal formation and bone structure, and mice with a mutation in Csf1 or Csf1 receptor have developmental deformities (46–48). Macrophages remove the wave of apoptotic cells during the formation of digits (49). They assist in angiogenesis and regulate blood vessel formation (50, 51).

In the adult, resident macrophages continue to play a critical role for many homeostatic functions that differ between tissues, which may explain why tissue macrophage expression profile varies between tissues (3, 52). Osteoclasts maintain bone development by constant bone resorption, and the predominant phenotype in mice that lack Csf1 or its receptor is osteopetrosis (53). Lung macrophages clear surfactant proteins in the lung, and in the absence of Csf2, which is required for macrophage maintenance and function, both mice and humans develop alveolar proteinosis (29, 54, 55). In the spleen, phagocytosis of red blood cells by red pulp macrophages controls iron levels and erythrocyte turnover (56). Microglia are important for neuronal development, and during the first few weeks of life they are involved in synaptic pruning (57, 58).
In the mature brain, microglia constantly survey the surrounding area, making transient synaptic connections with their long processes so that they are able to respond rapidly to injury and ischemia (59, 60). Intestinal macrophages constantly sense commensal microbes and sample luminal antigens by projecting their dendrites through intestinal epithelial cells (61, 62). They produce anti-inflammatory cytokines (63–65) critical for the local expansion of T-regulatory cells (66). Macrophages also clear bacteria that penetrate the epithelial barrier (67) and help contain pathogens locally by preventing their systemic dissemination (68).

Developmental niches rely on macrophages for maintenance. In the bone marrow, CD169+ macrophages maintain functional erythroblasts and contribute to the maintenance of late erythroid development (69). Bone marrow macrophages expressing VCAM1 are critical for effective recovery from irradiation and bone marrow transplantation as well as acute blood loss or hemolytic anemia. Their depletion conversely helps reduce hyperactive erythropoiesis in a polycythemia vera mouse model (69, 70). Bone marrow macrophages also maintain hematopoietic stem progenitor cell (HSPC) niches. Upon macrophage depletion, Nestin+ stromal cells that surround HSPC showed loss of many genes, such as CXCL12 and VCAM1, which are critical for HSPC maintenance and retention in the bone marrow leading to HSPC egress to the blood circulation (71). An important role may exist for macrophages in signaling and maintenance of other local stem cells niches such as in intestinal crypts (72, 73), although this has yet to be shown.

Macrophages express a wide range of cell surface and cytosolic receptors that allow them to detect tissue damage or infection (74). In the steady state, macrophages phagocytose and clear dying cells, and expression of oxidized phosphatidylserine on the surface of apoptotic cells constitutes a major signal for phagocyte engulfment. Macrophages produce opsonins such as milk fat globule EGF 8 (MFG-E8), Del-1, and growth arrest–specific gene 6 (Gas6) that bind to phosphatidylserine and promote engulfment through engagement of integrins or the Tyro3-Axl-Mer receptor tyrosine kinases (75–77). MFG-E8 release and the engagement of the Tyro3-Axl-Mer family receptor tyrosine kinases inhibit the induction of an innate immune response against self-antigens. Conversely, mice deficient in these pathways develop autoimmune and persistent inflammation (77, 78). Another mechanism of regulation of macrophage phagocytosis is through CD47, the ligand of the inhibitory SIRPα receptor on macrophages. Macrophages phagocytose red blood cells and foreign bodies that lack or downregulate this ligand (79).

Macrophages in the steady state maintain homeostasis by a wide array of housekeeping functions that clear unwanted debris and maintain the critical balance of inflammatory and tolerant signals.

**Disease**

Macrophages play a key role in acute inflammation following infection or tissue injury, as the classic signs of acute inflammation (rubor, calor, dolor, and tumor) can be attributed to processes they initiate (80). Macrophages recognize foreign antigens through a range of pathogen sensors that lead to the production of inflammatory cytokines and antimicrobial mediators (81). Inflammatory cytokines such as TNF-α, IL-6, and IL-1β lead to increased endothelium permeability and promote early recruitment of innate immune cells such as neutrophils and monocytes that differentiate locally into inflammatory macrophages. In parallel, resident dendritic cells leave the tissue and migrate in large numbers to the draining lymph node to initiate adaptive immune responses (4). Macrophages can also load extracellular antigen on MHC class II compartments and contribute to the differentiation of regulatory and effector CD4+ T cells. In addition to their contribution to effector immunity, macrophages are also essential in resolving inflammation by undergoing apoptosis, inducing an anti-inflammatory response, and facilitating wound-healing and repair mechanisms (16, 82).

**Macrophages in cancer.** As an emerging hallmark of cancer, inflammation paves the way for tumor growth, and chronic inflammation can give rise to tumors (83, 84). Solid tumors are infiltrated by a large number of immune cells among which macrophages represent the predominant population. Initially thought to play a role in antitumor immunity, studies in mice and humans have revealed the major protumorigenic role played by tumor-associated macrophages (TAM; refs. 85, 86). The protumorigenic roles of TAM result from macrophages attempting to restore tissue integrity by the promotion of an angiogenic program for tissue remodeling that also favors tumor growth, progression, and metastasis, and for the induction of immunosuppressive microenvironments that inhibit antitumor cytotoxic T-cell responses (2, 86, 87).

Macrophage phagocytosis is also important in regulating tumor progression. Despite their protumorigenic roles, depletion of macrophages before tumor induction leads to unchecked primary tumor growth and decreased survival (88, 89). Tumors upregulate CD47, the "don't eat me" signal, to inhibit phagocytosis of macrophages expressing SIRPα (90, 91), and inhibiting CD47 can increase phagocytosis of tumor cells (92). Macrophages are thus critically involved in both tumor regression and tumor spread.

**Macrophage heterogeneity in tumors.** The use of nonspecific cell markers as surrogates of macrophage function likely contributed to some of the confusion in our understanding of macrophage contribution to tumor outcome. Thus, it is important to avoid using single cell surface markers to identify macrophage populations and to interpret with caution those studies using suboptimal macrophage markers. In the tumor environment, in particular, precise identification of macrophage populations is necessary as many immune cells accumulate at the tumor site and in the tumor, and they can express heterogeneous markers. Identifying macrophages by CD68 alone has led to mixed assessment of the accumulation and the role of macrophage in tumors. Many studies show CD68 as correlating with tumor growth and decreased survival (85, 93). Other studies of human tumors, such as non–small cell lung carcinoma and colorectal cancer, show CD68+ cell accumulation as a good prognostic sign and correlated with increased survival (94, 95).
In addition to the heterogeneity of the population identified by using a single cell marker, the identification of a mix of pro- and anti-inflammatory signatures within the tumor microenvironment (96, 97) may reflect a spectrum of macrophage activation and responses by different macrophage populations that accumulate within the tumor. Macrophages at different stages in tumor development may act differently; the resident macrophages and monocyte-derived inflammatory macrophages may play diverse functional roles, and the nature of the tumor tissue likely drives different types of macrophage effector function locally. Better identification of macrophages at the tumor site through the use of multiple surface markers and differentiating subpopulations based on ontogeny will help clarify the roles of macrophages in cancer.

Macrophages and cancer therapy. The increased understanding of the key role played by Csf1 and its receptor in the promotion of macrophage survival and function has led to the development of novel drug targets that inhibit Csf1 receptor or its signaling in vivo. Both a small-molecule inhibitor of Csf1 receptor phosphorylation and monoclonal antibodies to Csf1r inhibit accumulation of infiltrating macrophages in the tumor (98, 99) and in combination with VEGF receptor-2 (VEGFR-2) blockade (100), chemotherapy (101), or radiotherapy (102) help reduce tumor growth in mice (103). Studies of macrophage phagocytic receptors have led to the development of high-affinity SIRPα variant monomers that inhibit CD47 and together with tumor-destroying antibodies showed an increase in survival and tumor regression (104). Similarly, blockade of the MFG-E8 cooperates with antitumor therapy to increase apoptosis and decrease tumor size (105). The effects of blocking recruitment of infiltrating monocytes to the tumor has also been explored as a therapeutic option by many mechanisms including Ccl2 blockade in breast cancer cell lines (87), VCAM1 inhibition (106), the use of GM-CSF antagonists in a genetic model of pancreatic tumors (107, 108), and a small-molecule inhibitor in neurofibroma (109). These results suggest that modulation of the macrophage and monocyte compartment can become an important component of an antitumor therapeutic regimen.

Regulation of macrophage function
The tumor model, alongside other inflammatory processes, drives macrophage action to both tolerance and activation. Macrophages can display a spectrum of phenotypes (110), and the transcriptional response to activation is controlled by different transcription factors and epigenetics (111). During macrophage development, master transcription factors such as Pu.1 bind to many regions throughout the genome along with the more specific CAAT/enhancer binding protein α (C/EBPα). These transcription factors open the chromatin, acting as master transcription factors that determine lineage specificity and remain stable in the presence of stimuli (112–114). These factors are important for cell type–specific differentiation; recently, Trib1 was shown to be important for the differentiation of splenic red pulp and inflammatory adipose tissue macrophages, likely mediated through altering the expression of CEBPα (115). A second level of transcription factors responds to the environment and further shapes the chromatin state. Although many genes are marked by master transcription factors as poised for activation, not all of them are transcribed. Inflammatory genes are kept in check in the steady state by binding of the transcriptional repressor Bcl-6 (116), the repressors NCoR and SMRT (117), and are associated with repressive histone marks such as H4K20me3 (118). Toll-like receptor activation induces the transcription factor NF-κB to bind to these regions and activates an inflammatory profile. However, the response to the stimuli is set during development and is cell type specific (119); for example, in fibroblasts exposed to the same stimuli as macrophages, the IL-12 locus instead of being activated remains inhibited and repressive methylation increases (120). A complex process of regulation controls the heterogeneous action of macrophages in development, inflammation, and tumor. Understanding the epigenetic level of regulation will provide further insights into how ontogeny and the environment shape the macrophage response.

Conclusions
Innate immunity comprises immune cells that are able to recognize a wide variety of stimuli and respond only after the triggering of robust inflammatory signals. Macrophages exemplify the balance that is required between tolerance and inflammation. In tumors, the dichotomy is particularly evident as self-tissue gone awry. Macrophages at these locations exhibit a combination of inflammatory and tolerance-inducing phenotypes. A better understanding of cellular ontogeny, tissue regulation, and epigenetics can clarify the function and response of different cell types. Ideally, this will lead to the development of more specific and effective therapeutics.

Authors’ Contributions
Conception and design: Y. Lavin, M. Merad
Writing, review, and/or revision of the manuscript: Y. Lavin, M. Merad

Received August 7, 2013; accepted August 19, 2013; published online October 7, 2013.

References
Macrophages: Gatekeepers of Tissue Integrity


Lavin and Merad

208 Cancer Immunol Res; 1(4) October 2013 Cancer Immunology Research


59. Lavin and Merad


Macrophages: Gatekeepers of Tissue Integrity

Yonit Lavin and Miriam Merad


Updated version
Access the most recent version of this article at:
http://cancerimmunolres.aacrjournals.org/content/1/4/201

Cited articles
This article cites 120 articles, 43 of which you can access for free at:
http://cancerimmunolres.aacrjournals.org/content/1/4/201.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerimmunolres.aacrjournals.org/content/1/4/201.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.