Origin and Functions of Tissue Macrophages

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Abstract

Macrophages are distributed in tissues throughout the body and contribute to both homeostasis and disease. Recently, it has become evident that most adult tissue macrophages originate during embryonic development and not from circulating monocytes. Each tissue has its own composition of embryonically derived and adult-derived macrophages, but it is unclear whether macrophages of distinct origins are functionally interchangeable or have unique roles at steady state. This new understanding also prompts reconsideration of the function of circulating monocytes. Classical Ly6c\textsuperscript{hi} monocytes patrol the extravascular space in resting organs, and Ly6c\textsuperscript{lo} nonclassical monocytes patrol the vasculature. Inflammation triggers monocytes to differentiate into macrophages, but whether resident and newly recruited macrophages possess similar functions during inflammation is unclear. Here, we define the tools used for identifying the complex origin of tissue macrophages and discuss the relative contributions of tissue niche versus ontological origin to the regulation of macrophage functions during steady state and inflammation.

Introduction

The Phagocyte: Ilya Metchnikoff and the Historical Perspective

Macrophages were first discovered late in the 19th century by Ilya Metchnikoff and are evolutionary conserved phagocytes that evolved more than 500 million years ago (Cooper and Alder, 2006; Tauber, 2003). Metchnikoff combined both evolutionary and ontological perspectives, which is particularly relevant in light of recent findings that have revolutionized the macrophage field. In the 1960s, van Furth proposed that all tissue macrophages originate from circulating adult blood monocytes, which has been the prevailing view for the last 40 years despite evidence that tissue macrophages are independent of circulating monocytes (van Furth and Cohn, 1968; Volkman et al., 1983; Sawyer et al., 1982). However, in the last few years, a series of more definitive publications have drastically revised our understanding of macrophage origin by demonstrating that many resident tissue macrophages are in fact established during embryonic development and persist into adulthood independently of blood monocyte input in the steady state (Ginhoux et
al., 2010; Schulz et al., 2012; Yona et al., 2013; Hashimoto et al., 2013; Epelman et al., 2014; Guilliams et al., 2013; Jakubzick et al., 2013). Metchnikoff defined the embryonic establishment of macrophages in the starfish, which has no formal vascular system. Accordingly, he argued for inflammation independent of blood vessels, countering Julius Cohnheim’s duly influential claims that “there is no inflammation without blood vessels.” Thus, in the 21st century, we have come full circle to these debates with the reality that inflammation, even in organisms anatomically more complex than starfish, centrally involves macrophages but can occur through mechanisms that are both dependent and independent of monocyte recruitment from the vasculature. Self-sustaining resident macrophages already present in the tissue make this possible.

As our tools develop, we continue to refine our understanding of tissue macrophages and can now further delineate multiple distinct embryonically derived macrophage lineages. Several detailed review articles describing different aspects of macrophage ontogeny and function have been published (Sieweke and Allen, 2013; Davies et al., 2013a; Wynn et al., 2013). Although a novel concept in and of itself, the dichotomy between embryonically derived and adult-derived macrophage populations does not fully encapsulate the entire complexity and diversity found among tissue macrophages. Intriguingly, tissue macrophages of differing ontological origins coexist, and when assessed as a group, they perform specialized, organ-specific functions. The goal of this review is to highlight emerging questions in the field, such as to what extent macrophage ontogeny versus the tissue niche dictates a macrophage’s function. Are we missing critical insight by not parsing macrophage function on the basis of origin? To what extent is there redundancy between adult-monocyte-derived macrophages and different resident macrophage subsets? We will utilize this framework to discuss how subsets of tissue macrophages and circulating monocytes originate, how expansion of tissue macrophages is regulated when homeostasis is disrupted, and what functions can be ascribed to individual subsets and lineages.

**Understanding the Tools Used for Defining Macrophage Ontogeny**

During early gestation (embryonic day 6.5 [E6.5]–E8.5), macrophages are first observed and expand in the extraembryonic yolk sac during what is termed primitive hematopoiesis (Samokhvalov, 2014) (see Figure 1). At this stage in development, macrophages are the only “white blood cell” produced, because restricted progenitors in the yolk sac give rise only to macrophages and red blood cells. This limited hematopoietic cell diversity is reminiscent of the Drosophila immune system (Makhijani and Bruückner, 2012) and indicative of the conserved origin of the yolk-sac-derived macrophage lineage. Subsequently (E8.5–E10.5), definitive hematopoietic stem cells (HSCs) emerge from the aorta-gonad-mesonephros and give rise to all immune lineages. Beginning at E10.5, HSCs migrate to the fetal liver, which then serves as the major hematopoietic organ during the remainder of embryonic development. Only in the perinatal period do traditional bone marrow HSCs become the primary site of hematopoiesis and produce the full complement of immune lineages (Orkin and Zon, 2008).

During embryonic development, transcription factor usage and surface-marker expression differ between yolk-sac-derived and definitive-HSC-derived macrophages. Definitive HSCs
are completely dependent on the transcription factor MYB, whereas yolk-sac-derived progenitors develop independently of MYB (Schulz et al., 2012). Furthermore, during embryogenesis, yolk-sac-derived macrophages have a characteristic CX3CR1^{hi} F4/80^{hi}CD11b^{lo} expression pattern (Schulz et al., 2012; Yona et al., 2013; Hashimoto et al., 2013; Epelman et al., 2014). Unfortunately, many adult resident tissue macrophages that originate during embryonic development alter the expression of cell-surface markers as the animal matures, hindering the ability to precisely track macrophage populations. Clear examples include embryonically established tissue macrophages that express CX3CR1 during development (lung and peritoneal macrophages and liver Kupffer cells) but lose expression after birth (Yona et al., 2013).

Genetic fate-mapping techniques give us the ability to precisely identify and track different embryonic macrophage populations into adulthood, and when combined with parabiotic and adoptive-transplant studies, they give us the ability to discern the relationship between macrophages and circulating blood monocytes. Fate-mapping studies utilize the principle of genetic recombination to permanently label cells (and all subsequent progeny) on the basis of the recombination-induced expression of a reporter gene that is under the control of a constitutive promoter (typically Rosa26). Genetic techniques have evolved to include inducible systems, where temporally controlling recombination facilitates precise labeling of embryonic populations and tracking into adulthood. Below, we will discuss in detail experimental evidence that undergirds the recently revised view of macrophage origins and identify both the strengths and the limitations of each approach (see a summary of tools used for defining macrophage origin in Table 1).

The inducible runt-related transcription factor 1 (Runx1^{CreER})-based system takes advantage of the early expression of Runx1 in the yolk sac at ~E7.0, which occurs prior to the development of definitive HSCs (Samokhvalov et al., 2007). Labeling of E7.0 Runx1-expressing yolk-sac-derived macrophages demonstrates that they persist faithfully into adulthood as microglia. Skin Langerhans cells contain both yolk-sac-derived and fetal-monocyte-derived populations, but fetal-monocyte-derived macrophages have become the dominant lineage over time (Hoeffel et al., 2012; Ginhoux et al., 2010). Whereas early labeling techniques (prior to E7.5) enable specific tracking of yolk-sac-derived macrophages (such as microglia), labeling between E8.0 and E10.5 leads to recombination in both yolk-sac-derived macrophages and early definitive HSCs, which migrate to the fetal liver and give rise to blood monocytes and lymphocytes (Ginhoux et al., 2010; Samokhvalov et al., 2007). As a result, the ability of Runx1^{CreER} to differentiate between tissue macrophages and blood monocytes during later developmental stages becomes obscured.

The inducible Csf1r^{CreER} has also been used for fate-mapping approaches. Initially, it was observed that E8.5-labeled (yolk-sac-derived macrophages) seed virtually all developing tissues in the embryo and show variable persistence into adulthood (Schulz et al., 2012). Similar to Runx1^{CreER}, Csf1r^{CreER} used at E8.5 in a separate study achieved ~30% labeling of yolk-sac-derived macrophages in the embryo and persistent labeling of microglia at the same rate in adults, confirming the near exclusive yolk sac origin of these cells (Epelman et al., 2014). The only other adult organs that retained significant numbers of yolk-sac-derived macrophages that originated from E8.5 were the heart and liver, suggesting that the
persistence of early yolk-sac-derived macrophages might be more restricted than currently appreciated. However, given that yolk sac progenitors migrate to the fetal liver at later stages of development, the possibility exists that these progenitors can continue to give rise to adult tissue macrophages. Unlike using Runx1CreER, using Csf1rCreER to label at E8.5 did not result in labeling of adult circulating blood monocytes, suggesting that a longer window for fate-mapping studies might exist. Two important caveats should be highlighted. First, unlike Runx1CreER, Csf1rCreER is a transgene and might not report native gene expression in all conditions. Second, the precision of any tamoxifen-inducible system that requires temporal activation is limited because of the half-life of tamoxifen. For example, labeling early yolk sac progenitors at E8.5 could be confounded by the persistence of tamoxifen in tissue and labeling at later developmental stages, which underscores the importance of monitoring recombination not only in tissue macrophages but also in later HSC-derived cells.

Adult definitive HSCs transiently pass through a FLT3+ stage during differentiation into all lineages (myeloid, lymphoid, megakaryocyte, and erythroid cells), which can be exploited genetically (Flt3Cre) for labeling adult-definitive-HSC-derived cells (Boyer et al., 2011). What is clear is that Flt3-Cre+ blood monocytes do not replace Flt3-Cre− tissue macrophage populations, even over extended periods of time (Epelman et al., 2014; Hashimoto et al., 2013; Schulz et al., 2012). Interpreting the ontological origin of Flt3-Cre− macrophages must be handled with care because it might be tempting to assume that the entire population developed from yolk-sac-derived progenitors, but this remains unclear. During situations where there is high demand (after macrophage depletion), the efficiency of Flt3Cre− dependent recombination within myeloid cells is reduced in comparison to the steady state. This represents a general limitation of Cre-dependent lineage-tracing systems. For example, after adoptive transplant of E14.5 fetal-liver definitive HSCs into sublethally irradiated adult animals, it takes ~8 weeks for Flt3Cre recombination to reach steady state in circulating blood monocytes. Prior to 8 weeks posttransplant, large numbers of Flt3-Cre− blood monocytes differentiate into long-lasting resident tissue macrophages (Epelman et al., 2014). Therefore, during times of high monocyte demand, HSC-derived monocytes appear to originate in a FLT3-independent fashion (Flt3-Cre−).

Additional complexity surrounds the observation that during early embryonic development, <5% of phenotypically MYB-dependent macrophages (HSC derived) display Flt3Cre− dependent recombination (Epelman et al., 2014). On the basis of available data, these cells might be derived from fetal-liver monocytes. Therefore, it is possible that Flt3-Cre− tissue macrophages in the adult consist of a mixed population containing macrophages derived from both the yolk sac and fetal monocytes (Epelman et al., 2014) (see Figure 1). The ontological and functional relationship between Flt3-Cre+ versus Flt3-Cre− fetal monocytes and adult-monocyte-derived macrophages is undefined. Conversely, it might be tempting to assume that Flt3-Cre+ tissue macrophages are continually replenished by Flt3-Cre+ circulating blood monocytes. Adoptive transplant and parabiotic studies suggest that in the majority of organs, HSC-derived macrophages—except for gut CX3CR1hi macrophages, cardiac CCR2+ macrophages, and dermal macrophage populations—also exist autonomously from circulating blood monocytes (Hashimoto et al., 2013; Epelman et al., 2014).
2014; Yona et al., 2013; Jakubzick et al., 2013). Consistent with these observations, lung macrophages that reside in the alveolus develop shortly after birth from HSC-derived fetal monocytes but are not replaced by circulating adult monocytes during parabiosis studies (Guilliams et al., 2013; Jakubzick et al., 2013).

A limitation of current fate-mapping strategies is that during embryonic development, incomplete labeling of monocyte and macrophage populations is invariably obtained. In part, this represents a technical limitation related to both the duration and levels of CRE expression—rapidly dividing progenitors might be too short-lived to permit recombination. However, an intriguing alternative interpretation is that in addition to incomplete labeling, embryonic macrophage lineages other than those derived from fetal monocytes and yolk sac progenitors might exist. In support of this possibility, Rag1Cre lineage tracing has identified a novel lymphomyeloid progenitor population that precedes the development of definitive HSCs (Böiers et al., 2013). As the name implies, lymphomyeloid cells give rise to only lymphocytes and monocytes, but not megakaryocytes or erythrocytes. Lymphomyeloid progenitors develop at E8.5–E9.5 through a RAG1⁺ progenitor and migrate to the fetal liver. Detailed analysis of fetal-liver-derived macrophages suggests that this subset is outcompeted over time by RAG1-independent macrophages (derived from fetal monocytes and/or the yolk sac). At E14.5 microglia, skin and liver macrophages were not labeled, suggesting that RAG-1-derived macrophages were significantly lost by this time point (Böiers et al., 2013; Schulz et al., 2012). It would be interesting to determine whether RAG1-dependent macrophages persist into adulthood as long-lived tissue macrophages.

**Monocyte-Specific Origins and Functions**

In the adult, monocytes originate from definitive HSCs through a characterized differentiation program involving progressively further committed progenitors. The identification of the monocyte-macrophage dendritic cell (DC) progenitor (MDP) provided a developmental link between both DCs and monocytes within a common differentiation pathway (Fogg et al., 2006). Recently, a Ly6c⁺ monocyte-specific progenitor downstream of MDPs has been identified in the bone marrow and spleen. Although this progenitor displays proliferative capacity, unlike mature monocytes, it lacks phagocytic activity (Hettinger et al., 2013). Our current understanding of monocytes suggests that there are two principle subsets: (1) classical Ly6c⁺ monocytes, which appear to be directly descendent from Ly6c⁺ monocyte progenitors, and (2) Ly6c⁻ nonclassical monocytes, which differentiate from Ly6c⁺ monocytes through an Nr4al-dependent transcriptional program (Hanna et al., 2011; Hettinger et al., 2013; Yona et al., 2013) (Figure 2). These monocyte subsets are highly conserved and transcriptionally closely match human monocyte subsets (Ingersoll et al., 2010). In both humans and mice, a third monocyte subset has also been suggested; it resembles an intermediate population between classical and nonclassical monocytes on the basis of cell-surface markers and transcriptional analysis. However, the function of this subset has not been well investigated (Schmidl et al., 2014).

The biology of the nonclassical subset of monocytes is unusual in that these cells appear primarily to function within the vasculature itself. They “patrol” the vasculature by slowly crawling over the endothelium, even moving against the flow of blood, in a manner...
dependent upon LFA-1 integrin (Auffray et al., 2007). The purpose of this behavior has recently been elucidated. Patrolling, nonclassical monocytes have a key role in clearing damaged endothelial cells, thereby maintaining integrity of the vasculature (Carlin et al., 2013). Although some investigators have considered these monocytes to be vascular “macrophages,” they closely cluster with other monocytes and do not express the core, mature macrophage signature of mRNA transcripts, including Mer tyrosine kinase (Gautier et al., 2012b; Jakubzick et al., 2013).

Although they are often referred to as “inflammatory monocytes,” recent work indicates that Ly6ch high monocytes can enter resting tissues. In this capacity, they patrol extravascular tissues to pick up antigens for transport to draining lymph nodes, all the while remaining relatively undifferentiated rather than committing to macrophages or DCs (Jakubzick et al., 2013). However, in the context of inflammation, recruited monocytes differentiate to macrophages (Jakubzick et al., 2013). Thus, monocytes of both subsets carry out a patrolling function in resting mice—the classical monocyte subset patrols extravascular tissues, and the nonclassical subset patrols the intravascular space (Figure 2). This patrolling feature distinguishes monocytes from macrophages, which have a limited ability to emigrate in comparison to monocytes. Once in tissue, Ly6C^hi monocytes can take on many characteristics of Ly6C^lo monocytes (Arnold et al., 2007; Hilgendorf et al., 2014) and enter the lymphatics after such conversion (Qu et al., 2004) (Figure 2).

In summary, a combination of approaches have greatly informed our understanding of the diversity and complex ontological origins of resident tissue macrophages (see Figure 1). Each organ has its own particular composition of embryonically derived and adult-derived macrophage subsets, and each organ dictates the degree to which circulating monocytes replace resident macrophages after birth. As we continually refine our understanding of macrophage lineage, an important new goal is to determine the functions of these cells, both through direct targeting of individual macrophage subsets and through reflecting on prior studies and putting into context observations that were made prior to the revelation that embryonically derived macrophages exist.

The Developing Embryo and Macrophage Function

Perhaps the simplest way to learn what functions embryonically derived macrophages might possess in the adult is to first examine what these cells do during embryonic development. In this way, we can determine whether macrophage functions executed in the embryo are “hard wired” and specifically retained by these subsets in the adult. Mice deficient in PU.1, CSF1R, or M-CSF (CSF1) lack many tissue macrophages from the earliest stages of development beginning at the time of yolk sac hematopoiesis. These animals display increased perinatal mortality, reduced postnatal survival, and stunted growth (Dai et al., 2002; McKercher et al., 1996; Wiktor-Jedrzejczak et al., 1990). Careful examination of macrophage-deficient embryos has revealed defects in embryonic vascular development. Spi1^−/− and Csf1^op/op animals demonstrate reduced CNS vascular complexity and branching as early as E11.5 (Nucera et al., 2011; Fantin et al., 2010; Arnold and Betsholtz, 2013). Elegant experiments utilizing zebrafish and genetic mouse models have revealed that macrophages orchestrate CNS vascular development by acting as endothelial cell
chaperones promoting endothelial tip cell fusion in a VEGF-A-independent manner (Fantin et al., 2010). Intriguingly, macrophages found in the developing retina are required for blood vessel regression through a noncanonical WNT- and FLT1-dependent mechanism (Lobov et al., 2005), indicating that macrophages might interact with the developing vasculature in distinct ways depending on the tissue context.

In addition to regulating embryonic vascular growth and patterning, macrophages participate in other important processes during development. In the developing brain, microglia mediate neuronal patterning through regulation of neuronal survival and programmed cell death (Wake et al., 2013; Nayak et al., 2014). With respect to neuronal survival, microglia are essential for cortical layer V formation, where they release multiple growth factors, including IGF-1 (Ueno et al., 2013). Conversely, microglia pattern the developing hippocampus and cerebellum by triggering the death of excess neurons through DAP12 signaling and the release of reactive oxygen species (Marín-Teva et al., 2004; Wakselman et al., 2008). Microglia-induced neuronal cell death is accompanied by minimal inflammation and rapid phagocytosis of apoptotic neurons. Similarly, microglia play essential roles in synaptic patterning by using DAP12-, CX3CR1-, and complement-dependent mechanisms (Paolicelli et al., 2011; Roumier et al., 2004; Stevens et al., 2007). Recently, a more general role for embryonic macrophages in the clearance of senescent cells during embryonic development was described (Muñoz-Espín et al., 2013; Storer et al., 2013).

A common feature of Csf1op/op, Csf1r−/−, and Spi1−/− mice is defective long-bone formation, tooth eruption, and osteopetrosis (Dai et al., 2002; Wiktor-Jedrzejczak et al., 1990; Tondravi et al., 1997). Each of these phenotypes is a consequence of failed osteoclast development. Osteoclasts are terminally differentiated macrophage-derived cells with specialized functions in bone remodeling. With respect to long-bone formation, macrophage-deficient embryos display defects in growth-plate dynamics, fail to undergo timely endochondrial ossification, and contain increased numbers of hypertrophic chondrocytes (Ortega et al., 2010). Failed tooth eruption and osteopetrosis are a direct result of deficiencies in osteoclast-mediated bone demineralization and remodeling (Kodama et al., 1991). It is not entirely known from what macrophage lineage(s) osteoclasts are derived. In the adult mouse, parabiosis studies have revealed that monocytes can be recruited to the bone and differentiate to osteoclasts. By using a transgenic mouse expressing a photoconvertable GFP molecule (Kikume Green-Red), Kotani et al. (2013) identified a contribution of splenic monocytes to osteoclasts. This study did not employ genetic lineage-tracing techniques, and although adult monocytes can contribute to the osteoclast pool, it remains unclear to what degree and whether embryonically derived macrophages do as well. Moreover, the ontogeny of osteoclasts during embryonic and postnatal development remains unresolved.

Consistent with a role in tissue repair during development, a recent manuscript reported that neonatal macrophages are required for cardiac repair after neonatal myocardial infarction (Aurora et al., 2014). After cardiac injury, neonatal macrophages were required for angiogenesis and were associated with a less vigorous inflammatory response when compared to macrophages in the injured adult heart. Unfortunately, this study did not include a detailed characterization of monocyte and macrophage subsets or lineages. Prior
studies in humans have also described differences in inflammatory potential between fetal and adult mononuclear phagocytes after LPS stimulation (Caron et al., 2010; De Wit et al., 2003; Levy et al., 2004; Weatherstone and Rich, 1989). Together, these findings suggest that embryonic and neonatal macrophages play important roles in tissue remodeling during development, and compared to adult-monocyte-derived macrophages, they have a reduced capacity to generate inflammatory responses.

**Monocyte and Macrophage Dynamics and Function in Tissues**

Comprehensive transcriptional analysis of resident macrophages through the ImmGen Consortium suggests that transcriptionally, resident macrophages isolated from various organs are as different from each other as they are from circulating blood monocytes during steady state (Gautier et al., 2012b), suggesting that the tissue niche provides instructive signals orchestrating macrophage differentiation. However, because many resident macrophage populations consist of a mixture of embryonic- and adult-HSC-derived macrophages and because fate-mapping studies were not employed, we must view the ImmGen data with the understanding that lineage-specific functions among tissue macrophages were not strictly assessed (Epelman et al., 2014; Hoeffel et al., 2012; Schulz et al., 2012; Hashimoto et al., 2013). In this section, we will highlight the relevant studies that allow us to discern how different tissue macrophage populations change during times of stress, whether functions possessed by macrophages during embryonic development are “hard wired” and therefore retained by their adult progeny, and how macrophages are conditioned by the tissue in which they reside.

**Brain Microglia and the Synapse—A Dynamic Bidirectional Relationship**

As mentioned previously, unlike other macrophage pools, brain microglia are derived entirely from yolk sac macrophages; therefore, in the steady state, functions can be directly attributable to this lineage. After birth, a massive expansion of microglia cell numbers is driven exclusively through in situ proliferation via M-CSF and interleukin-34 (IL-34) without monocyte input (Wegiel et al., 1998; Wang et al., 2012; Ginhoux et al., 2010; Ajami et al., 2007). As in the embryo, microglia retain their ability to alter neuronal circuitry during the postnatal period, where they are critical for synaptic pruning through engulfment of neuronal synapses, in addition to phagocytosis of apoptotic neurons (Paolicelli et al., 2011). Specifically, Cx3cr1+/− mice have a transient reduction in microglia numbers during the early postnatal period, incomplete synaptic pruning, and decreased functional brain connectivity, resulting in neurodevelopmental abnormalities related to social interaction and repetitive behaviors (Zhan et al., 2014).

Microglia dynamically interact with neurons at both pre- and postsynaptic sites through the extension of microglial processes, where prolonged contact leads to elimination (Miyamoto et al., 2013). During normal visual stimulation, microglia respond to light-dark cycles by changing the frequency and duration of contacts with multiple synapse-associated elements (Tremblay et al., 2010). Interestingly, contact (and envelopment) by microglia is correlated with subsequent changes in synaptic connections, suggesting that sensory experiences manipulate microglia activity acutely, and microglia are involved in the modulation of sensory processing by regulating synapses. Their role in the steady-state adult brain can be
ascertained through novel genetic techniques that now allow for specific deletion of brain microglia in the absence of the confounding effects of surgical trauma with the use of the Cx3cr1CreER system. Specific microglia deletion results in decreased synaptic formation and learning (Parkhurst et al., 2013). Loss of microglia-specific brain-derived neurotrophic factor produces a similar phenotype, suggesting that a key steady-state function of microglia is promoting synaptic plasticity of neurons in response to learning cues secondary to the release of soluble mediators. During brain ischemia, microglia significantly increase the duration of their interactions with synapses, which correlates with the loss of dysfunctional synaptic structures and suggests that microglia serve an early protective role after brain injury (Wake et al., 2009).

**Microglia and Recruited Monocytes during Brain Injury**

Autoimmune experimental encephalitis (EAE) in the mouse is similar to human multiple sclerosis and is believed to depend on the activation of myelin-specific T cells that trigger resident microglia and monocyte-derived macrophages, inflammatory demyelination, and a relapsing and remitting motor paralysis. Although this has been well investigated, we highlight two studies in which the roles of resident microglia and recruited monocytes were separated. Through the elegant use of mixed parabiotic and bone-marrow-transplant studies, specific infiltration of monocyte-derived macrophages was both correlated to and required for progression of neurological disease, indicating a critical role for infiltrating monocytes in the pathogenesis of EAE (Ajami et al., 2011). Utilizing the Cx3cr1CreER system, a recent study has demonstrated that specific deletion of the kinase TAK1 in microglia (and not infiltrating monocytes) prevents both initial and relapsing paralysis, as well as inhibition of cellular expansion and disease progression (Goldmann et al., 2013). Together, these data suggest that both lineages are required for EAE progression and that early microglia activation via TAK1 is necessary for disease initiation, whereas the latter monocyte infiltrate (which is dependent on microglia activation) drives neurological damage through demyelization and progression of paralysis. As in EAE, if monocytes are prevented from infiltrating the brain after ischemic injury, the permeability of the blood-brain barrier is reduced and infarct size is reduced, suggesting a pathological role for monocyte-derived macrophages during ischemia (Dimitrijevic et al., 2007).

Whether or not monocytes enter the CNS is a difficult question to address because it depends on the techniques utilized. For example, with parabiosis, acute motor nerve damage leads to microglia expansion that occurs without chimeric monocyte input. However, if peripheral chimerism is achieved through irradiation and intravenous injection of large numbers of bone marrow cells (standard bone-marrow-transplant studies), chimeric blood monocytes are recruited to the sites of neuronal injury (Ajami et al., 2007). Use of irradiation is a key confounder in microglia dynamics, given that there is disruption of the blood-brain barrier and entry of monocytes that would otherwise not enter into the brain in the setting of adoptive transplant (Mildner et al., 2011). During EAE, the microglia population expands locally through proliferation, whereas recruited monocytes are eliminated over time, suggesting that even during intense inflammation, monocyte-derived macrophages do not have the capacity to become self-sustaining microglia and have little long-term access to the CNS (Ajami et al., 2011). Although many homeostatic functions of
yolk-sac-derived microglia are under intense investigation, what has not been specifically addressed is the relative ability of monocyte-derived macrophages to perform highly specialized functions typically associated with microglia, such as synaptic pruning. Monocyte-derived macrophages are found in the CNS several months after EAE induction, and it would be interesting to observe whether these cells, even though their presence is temporary, are able to take over homeostatic functions in brain areas in which they reside.

Neurodegenerative diseases are chronic disorders often associated with aging and numerical expansion of microglia. During the aging process, microglia acquire an irregular shape, occupy smaller territories, and form inclusions containing apparent synaptic elements, suggesting that dysfunction develops in macrophage degradative pathways with age (Tremblay et al., 2012; Wake et al., 2013). Similarly, in animal models of Alzheimer’s disease, microglia numerically expand and progressively accumulate beta amyloid plaques, which is believed to impair phagocytic function and allow beta amyloid plaques to accumulate in the brain, suggesting that phagocytosis of plaques could be protective. SNPs in genes encoding CD33 (a phagocytosis inhibitor) or TREM2 (which mediates neuronal uptake) are associated with Alzheimer’s disease, lending clinical credence to the idea that impaired phagocytic clearance of plaque promotes disease progression (Griciuc et al., 2013; Guerreiro et al., 2013). Multiple studies have suggested that recruitment of CCR2+ (Ly6c^hi) monocytes improves clearance of plaque depositions, improves cognitive function, and decreases mortality in animal models of Alzheimer’s disease, suggesting a clear protective role for infiltrating monocytes (Naert and Rivest, 2011; El Khoury et al., 2007). However, the use of irradiation and bone-marrow-transplantation models to localize CCR2 deficiency to the bone marrow compartment, which, as stated above, might allow circulating monocytes access to the brain, suggests that formal proof that monocytes enter the CNS in Alzheimer’s models is currently lacking. In fact, there is strong evidence that circulating monocytes do not enter the brains of other neurodegenerative disorders (such as Huntington’s disease), but as of yet, the role of microglia in Huntington’s disease is not clear (Ajami et al., 2007). Understanding the role of monocytes and microglia in neurocognitive dysfunction is challenging because subtle dysfunctions might not be readily detectable. For example, Cx3cr1^−/− mice have been widely utilized over the last decade in multiple fields, and as mentioned above, we have only recently identified neurocognitive deficits related to microglia in this strain (Zhan et al., 2014). Together, these data demonstrate that resident microglia have important homeostatic functions in regulating synaptic development, and the diseased state (e.g., Alzheimer’s disease) might be insufficient to prevent chronic neurodegeneration. Taking advantage of macrophage plasticity in the setting of dysfunctional brain microglia by promoting monocyte entry could lead to novel therapeutic approaches for neurocognitive disorders.

**Cardiac Macrophages—Ontologically and Functionally Distinct Subsets**

Unlike the brain, which exclusively contains yolk-sac-derived microglia, the heart contains several discrete macrophage populations with mixed ontological origins (Epelman et al., 2014). CCR2^− macrophages are largely derived from embryonic progenitors, including contributions from yolk sac macrophages, and are primarily autonomous from blood monocytes. In contrast, resident cardiac CCR2^+ macrophages are derived entirely from
definitive HSCs and are replaced slowly by blood monocytes. In the setting of acute hypertensive stress, both resident embryonically derived macrophage populations and recruited populations expand by proliferation in a manner analogous to that of peritoneal macrophages during sterile inflammation (Davies et al., 2013b). Functionally, when all cardiac macrophages are depleted after ischemic injury, a net beneficial role in scar formation is revealed, given that macrophage-depleted mice demonstrate poor infarct healing and myocardial rupture (van Amerongen et al., 2007; Nahrendorf et al., 2007). Subset-specific transcriptional profiling indicates that CCR2\(^+\) macrophages differentially express multiple components of the NLRP3 inflammasome and are required for IL-1\(\beta\) production, suggesting that adult-derived macrophage lineages within the myocardium might be primed to generate inflammatory responses (Epelman et al., 2014). These data could help explain why targeting CCR2\(^+\) macrophages is beneficial after ischemic injury (Frangogiannis et al., 2007). Whether reparative and proangiogenic responses are mediated by adult and/or embryonically established macrophage populations remains unknown.

### Depletion, Plasticity, and Function of Tissue Macrophages

After depletion of cardiac macrophages, circulating Ly6\(^{c,hi}\) monocytes take up permanent residence within cardiac tissue and give rise to the dominant population of resident tissue macrophages. After repopulation is complete, autonomy of tissue macrophages is restored, albeit with a large complement of adult-monocyte-derived macrophages as the new resident macrophage population (Epelman et al., 2014). If Ly6\(^{c,hi}\) monocytes are absent from circulation (such as in Ccr2\(^{-/-}\) mice), resident macrophages are fully capable of repopulating the tissue niche (see Figure 3). Careful examination of the repopulation of tissue macrophages after depletion has revealed that multiple mechanisms are at play. As a result, a universally applied repopulation strategy does not exist, because both organ- and depletion-specific factors apply. For example, similar to depletion of cardiac macrophages, clodronate-liposome-mediated depletion results in profound and permanent loss of embryonically established resident tissue macrophages, including liver Kupffer cells and splenic red-pulp macrophages. Both Kupffer cells and splenic red-pulp macrophages are subsequently replacement by adult-derived (FLT3-dependent) monocytes, indicating that circulating monocytes have a competitive advantage over the remaining resident tissue macrophages (Epelman et al., 2014). These data indicate that forced “ontological reprogramming” of tissue macrophages can be easily achieved with clodronate-liposome-mediated depletion strategies in these organs. The functional significance of ontological reprogramming is unknown, and defining whether distinct macrophage lineages harbor unique or overlapping functions will require tissue-specific investigations.

Alveolar macrophages reside on the epithelial surface of the lung, and in contrast to other resident macrophage populations, they are in direct contact with the environment, which includes commensal bacteria, inhaled particulates, and host-epithelial-derived factors, such as surfactants. Embryonically derived fetal monocytes appear to colonize the lung shortly after birth and differentiate into alveolar macrophages in a GM-CSF-dependent process (Guilliams et al., 2013). GM-CSF signaling drives expression of PPAR-\(\gamma\), a characteristic feature of alveolar macrophages (Malur et al., 2011), which contributes to the unique gene-expression profile of lung macrophages (Gautier et al., 2012a). Alveolar macrophages
normally live independently of blood monocyte input; however, an interesting competition takes place if alveolar macrophages are depleted. CD163-DTR-mediated depletion results in repopulation by in situ proliferation independently of blood monocyte input (Hashimoto et al., 2013) (see Figure 3). However, if alveolar macrophages are depleted by genotoxic injury via lethal irradiation, recruited monocytes now gain the competitive advantage and repopulate the alveolar macrophage niche. Intriguingly, despite genotoxic injury, irradiated resident alveolar macrophages can re-expand if recruited monocytes cannot receive GM-CSF signals. Use of sublethal irradiation seems to level the playing field such that both resident macrophages and blood monocytes contribute to repopulation. Functionally, similar observations have also been seen in the heart (Epelman et al., 2014). These data indicate that most resident tissue macrophages maintain themselves indefinitely without monocyte input, but if an acute and profound depletion occurs, circulating monocytes can readily repopulate the macrophage niche.

Pulmonary surfactant is produced by alveolar epithelial cells, and together with other pathways, it acts to suppress alveolar macrophage activation during resting conditions (Hussell and Bell, 2014). An essential role of alveolar macrophages is the ability to phagocytose excessive surfactant proteins and prevent alveolar proteinosis (Dranoff et al., 1994; Guilliams et al., 2013). After alveolar macrophage depletion, local production of GM-CSF is essential for both alveolar macrophages and recruited monocytes to reverse alveolar proteinosis (Hashimoto et al., 2013), again highlighting the ability of the tissue niche to govern macrophage differentiation independently of developmental lineage.

Splenic red-pulp macrophages are required to take up senescent red blood cells and specifically utilize the transcription factor SPI-C to drive the genes necessary for iron uptake (Kohyama et al., 2009). Unlike other embryonically derived macrophages, splenic red-pulp macrophages receive instructive signals from the spleen to express SPI-C, highlighting clear tissue-specific steady-state functions. In the setting of hemolysis, excessive heme induces the loss of resident red-pulp macrophages. Circulating monocytes enter the spleen and upregulate SpiC, leading to macrophage differentiation, reestablishment of the splenic red-pulp macrophage compartment, and restoration of iron homeostasis. These data provide further evidence that local signaling within tissues might be sufficient to promote the acquisition of specialized macrophage functions irrespective of the ontological lineage (Haldar et al., 2014).

Splenic marginal-zone macrophage development is dependent on the nuclear receptor LXR and functions to limit bloodborne infections (A-Gonzalez et al., 2013). Although the precise origin of marginal-zone macrophages is not known, these cells proliferate in the first weeks of life. LXR-deficient mice have normal monocyte numbers, and adoptive transfer of wild-type monocytes into LXR-deficient mice leads to functional reestablishment of the splenic marginal-zone macrophage niche, indicating that monocytes are instructed within the spleen in a LXR-dependent manner to acquire marginal-zone macrophage fate. Together, these data highlight the ability of the spleen to drive monocyte-to-macrophage differentiation and compartmentalize monocyte fate to specific splenic macrophage subsets (red pulp versus marginal zone).
However, it remains unclear whether the tissue microenvironment is sufficient to induce tissue-specific macrophage mRNA transcripts in organs beyond the spleen. GATA6 is a transcription factor that selectively regulates peritoneal macrophage transcripts (Gautier et al., 2014; Gautier et al., 2012b; Okabe and Medzhitov, 2014; Rosas et al., 2014). In the absence of GATA6, the major resident macrophage population in the peritoneum is greatly contracted, but a second, smaller population that normally lacks GATA6 expression remains at normal frequency (Gautier et al., 2014; Okabe and Medzhitov, 2014; Rosas et al., 2014). Enhanced apoptosis in the resting state (Gautier et al., 2014) and a failure to proliferate in states of inflammation (Rosas et al., 2014) explain the loss of macrophages. GATA6 can be induced by vitamin A signaling, but addition of vitamin A to cultured macrophages will induce GATA6 only when sources of fetal macrophages, such as fetal-liver macrophages, are used (Okabe and Medzhitov, 2014). A working model is that mature macrophages have undergone epigenetic changes that prevent such induction, such that GATA6 has not yet been demonstrated to be induced in monocytes or other macrophages from adult mice. In line with this model, GATA6 is not induced in monocyte-derived macrophages recruited to the peritoneum during thioglycollate-elicited inflammation. These elicited macrophages upregulate canonical, universal macrophage differentiation transcripts, but they induce only some peritoneal-cavity-selective transcripts (Gautier et al., 2012b). It will be important to determine in the future whether the ablation of resident peritoneal macrophages provides a niche in which recruited monocytes can replace them with induction of the full panoply of local resident macrophage transcripts, including GATA6 and its targets.

**Macrophages and Tissue Regeneration**

One of the most interesting aspects of macrophage function is their involvement in tissue repair and regeneration. Model organisms, including salamanders, zebrafish, and neonatal mice, all require macrophages for tissue regeneration; however, no studies have addressed the contribution of distinct macrophage subtypes and/or lineages in this process (Godwin et al., 2013; Aurora et al., 2014; Huang et al., 2013). The liver is the best characterized adult organ capable of regeneration in mammals after injury or resection. Macrophage expansion occurs in the setting of injury, and a significant component occurs through monocyte recruitment, although specifics regarding the contribution of resident versus recruited cells are lacking. After injury, liver macrophages engulf hepatocyte debris and activate a program leading to the secretion of WNT ligands, which direct hepatocyte differentiation from progenitors (You et al., 2013; Boulter et al., 2012). Although macrophage depletion impairs hepatocyte differentiation, bile duct regeneration is not affected, highlighting the potential specificity of macrophage-mediated regeneration. In the absence of circulating monocytes (such as in CCR2- or MCP-1-deficient mice), fibrosis after acute liver injury or diet-induced nonalcoholic liver disease is reduced, suggesting that monocyte-derived macrophages rather than Kupffer cells promote liver fibrosis (Kassel et al., 2010; Karlmark et al., 2009). The ability to induce fibrosis at the site of injury appears to be a common function of recently recruited monocytes, not only in the liver but also in the heart and kidney, suggesting a conserved transcriptional program (Kaikita et al., 2004; Kitagawa et al., 2004). Studies that focus on the role of monocyte-derived macrophages invariably target the CCR2 pathway, which also limits monocyte egress from the bone marrow during steady state. CCR2 deficiency serves as our best model of peripheral monocyte ablation, but several important
caveats must be taken into account. CCR2 is also required for HSCs to traffic to sites of injury (such as the liver), and CCR2 signaling in nonimmune cells might also promote injury (Si et al., 2010; Zhou et al., 2006).

Given recent advances in our understanding of macrophage ontogeny, novel genetic tools for and approaches to separating out functions of infiltrating monocytes from resident embryonically derived and adult-derived macrophages are being developed. Future studies will focus on deciphering the function of individual pathways (such as TAK1 in microglia) within distinct macrophage lineages. Common macrophage functions, such as phagocytosis, could be easily complemented by nonresident monocyte-derived macrophages. Whether specialized macrophage functions are specific to resident macrophage lineages or can be fully acquired by recruited, monocyte-derived macrophages remains an important question and will require careful examination.

**Monocyte and Macrophage Proliferation in Tissues**

Macrophage expansion in tissues during disease is common and critically modulates injury versus healing. However, the contribution of monocyte recruitment and local proliferation is only now coming to light. Until the demonstration that proliferation of macrophages could solely account for macrophage expansion at sites of nematode infection, macrophage proliferation was not recognized as a powerful modifier of macrophage numbers within organs. IL-4 signals serve in this context as a major driver of proliferation, raising the initial idea that macrophage proliferation is a particularly specialized feature of T helper 2 (Th2)-cell-oriented immunity (Jenkins et al., 2011; Jenkins et al., 2013). Cardiac injury and sterile peritonitis induce monocyte recruitment and subsequent proliferation in tissues of resident macrophages, recruited monocytes, and monocyte-derived macrophages (Davies et al., 2013b; Epelman et al., 2014). Recent studies of adipose tissue and atherosclerosis suggest that macrophage proliferation contributes to inflammation in contexts beyond Th2 cell immunity. Understanding whether monocyte recruitment or proliferation of resident and recruited macrophages drives expansion is critical if therapies targeting macrophages are to move forward. For example, if local macrophage expansion is sufficient to mediate pathology, blockade of monocyte recruitment would be expected to only modestly affect disease progression.

Recruitment and proliferation appear to be intertwined. Macrophage proliferation in adipose tissue has been reported to be dependent on MCP-1, as is monocyte emigration from bone marrow and entry into circulation and peripheral tissue (Serbina and Pamer, 2006; Amano et al., 2014). As opposed to the idea that MCP-1-CCR2 signaling in adipose tissue macrophages drives proliferation directly, it seems more likely that proliferation of macrophages is coupled to signals downstream of CCR2-mediated monocyte recruitment. The concept of coupled proliferation and recruitment also extends to atherosclerosis. There is significant evidence that early in disease progression, recruited monocytes enter into plaque, take up cholesterol, and become dysfunctional foam cells. Blockade of monocyte recruitment prevents disease progression (Randolph, 2014). However, recent data also generate a compelling case for proliferation as a major determinant for macrophage burden within plaques (Robbins et al., 2013).
What is clear is that recruited monocytes, monocyte-derived macrophages, and resident macrophages utilize proliferation as a strategy to control cell numbers within tissue. Given that monocyte numbers can be augmented simply through additional recruitment, the reader might ask, why do monocytes and monocyte-derived macrophages proliferate in tissues? One possibility is that local expansion and contraction could be a more efficient and tightly regulated process. If local expansion (of resident macrophages) is insufficient, increased production and release of monocytes from bone marrow and splenic stores could be utilized (Swirski et al., 2009). However, as a generalized mechanism, increased peripheral monocyte production and release in the hopes that those short-lived cells rapidly and specifically enter an inflamed tissue appears less efficient. The very recent identification of proliferating Ly6c<sup>+</sup> monocyte progenitors in the spleen and bone marrow might reveal another nuanced mechanism in which injured tissue recruits monocyte progenitors that locally proliferate and thus give the tissue an opportunity to control monocyte numbers in situ rather than through recruitment. Monocyte recruitment and local proliferation are unlikely to be independent mechanisms. In fact, the currently available evidence supports a model in which recruitment of monocytes is linked to proliferation of previously recruited monocyte-derived macrophages (Randolph, 2013).

Human Correlates to Ontologically Distinct Tissue Macrophage Subsets

Few studies have examined whether human tissue macrophages are similarly composed of resident embryonically derived and monocyte-derived macrophage subsets. Instead, designation of macrophage populations in humans has largely been focused on defining cell types on the basis of the M1 (interferon-γ-mediated) versus M2 (IL-4-mediated) macrophage phenotype. The M1 versus M2 designation is a widely utilized paradigm in mice and appears to be conserved to some degree in humans (Martinez et al., 2013). However, a recent study has demonstrated that treatment of human blood monocytes with a variety of stimuli results in acquisition of a spectrum of macrophage phenotypes that span between classic M1 and M2 definitions, casting doubt on the concept that M1 versus M2 behavior can be used for defining macrophage subsets (Xue et al., 2014; Murray et al., 2014, in this issue of Immunity).

Studies focusing on human skin after HSC transplantation have revealed indirect evidence that tissue macrophages are long lived and might exist independently of circulating progenitors. Careful examination of skin HLA-DR<sup>+</sup> myeloid cells from patients receiving sex-mismatched bone marrow transplants demonstrated that DC populations reached nearly 100% chimerism within 40 days posttransplant, indicating a clear dependency on circulating cells (Haniffa et al., 2009). However, ~25% of skin macrophages were not replaced, even after 1 year posttransplant, suggesting that some human skin resident macrophages might persist independently of circulating monocyte input, which is similar to what has been observed in mice (Jakubzick et al., 2013; Tamoutounour et al., 2013).

Because genetic lineage tracing is not feasible in humans, the optimal strategy for identifying and defining distinct macrophage lineages in human tissue is not immediately obvious. Identification of conserved gene-expression signatures represents one potential solution to this problem. Systematic analysis of the ImmGen data set from C57BL/6 mice has
yielded the discovery of a core macrophage gene-expression signature, allowing the
distinction of monocytes, macrophages, and DCs (Gautier et al., 2012b). Major surface
proteins found in the core signature include Mer tyrosine kinase and FcgRI, molecules that
themselves highlight the specialized function that macrophages have in engulfing apoptotic
cells and responding to immunoglobulins made during adaptive immunity to coordinate
tissue responses, respectively. Importantly, Mer tyrosine kinase and FcgRI were recently
shown to also be conserved in human monocyte-derived macrophages (Xue et al., 2014).
With this methodology, it is conceivable that by defining the core signatures of
embryonically derived and monocyte-derived macrophage subsets in mice, it might be
possible to identify corresponding macrophage populations in humans.

Summary

There are clear examples in which tissue-specific factors drive highly specialized
macrophage functions irrespective of their ontological origin, suggesting tremendous
plasticity and redundancy in the mononuclear phagocyte system. Whether embryonic and
adult macrophages possess specialized roles has yet to be formally tested. However, our
conceptual understanding and genetic tools are now sufficiently developed to precisely
follow both embryonic and adult macrophage subsets in health and disease, which should
allow important and unanswered questions in the field to be addressed (see Table 2). In
order to develop novel therapies, a critical future goal is to harness this newfound
understanding that different macrophage lineages exist within tissues and clarify whether
these distinct lineages differentially contribute to tissue damage and repair.

REFERENCES

A-Gonzalez N, Guillen JA, Gallardo G, Diaz M, de la Rosa JV, Hernandez IH, Casanova-Acebes M,
Lopez F, Tabraue C, Beceiro S, et al. The nuclear receptor LXRα controls the functional
Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia
18026907]
Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE
progression, but do not contribute to the resident microglia pool. Nat. Neurosci. 2011; 14:1142–
1149. [PubMed: 21804537]
Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, Shen Y, Czech MP, Aouadi
M. Local proliferation of macrophages contributes to obesity-associated adipose tissue
Arnold T, Betsholtz C. The importance of microglia in the development of the vasculature in the
Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory
Geissmann F. Monitoring of blood vessels and tissues by a population of monocytes with patrolling
Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R, Sadek HA, Olson EN.
[PubMed: 24569380]


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Figure 1. Macrophage Lineages, Ontogeny, and Contribution to Populations of Resident Tissue Macrophages

(A) Schematic depicting the origins of embryonic and adult macrophage lineages. Genetic lineage tracing using Flt3Cre and direct labeling techniques, including Csf1rCreER and Runx1CreER, allow identification of adult-monocyte-derived and yolk-sac-derived macrophages. There are no available tools for lineage tracing of fetal-liver-monocyte-derived macrophages only.

(B) Contribution of macrophage lineages to populations of adult resident macrophages. HSC-derived populations include embryonic populations and no definitive evidence of yolk sac origin (embryonic), whereas HSCs (adult) have passed through a FLT3+ stage (Flt3-Cre+) and are continually replaced by circulating adult monocytes.
Figure 2. The Life Cycle of Monocyte Subsets and Macrophages in Resting and Inflamed Tissues

In the vasculature, classical Ly6<sup>hi</sup>CD43<sup>−</sup> monocytes give rise to nonclassical Ly6<sup>lo</sup>CD43<sup>+</sup> monocytes through an NR4A1-dependent manner (dashed black arrow). Nonclassical monocytes go on to patrol the intravascular endothelial cell surface to clear dying endothelial cells. Classical monocytes can exit the bloodstream and have the capacity to patrol extravascular tissues. These tissue monocytes can transport antigens to lymph nodes with minimal differentiation changes from their state in blood, although a proportion can convert to Ly6<sup>lo</sup> monocytes. However, in the context of inflammation, classical monocytes readily differentiate to macrophages. These monocyte-derived macrophages are distinct from resident macrophages, which in many organs are embryonically derived and capable of sustaining themselves by local proliferation. Both recruited and resident macrophages share the capacity for proliferation in tissue during inflammation.
Figure 3. Schematic Representation of Repopulation Strategies Used by Tissues to Replenish Macrophage Numbers via Different Depletions Methods

(A) Cardiac macrophages. In the steady state, the majority of cardiac macrophages are embryonically derived (red, Flt3-Cre−), and a smaller contribution is derived from HSCs (green, Flt3-Cre+). After depletion, Ly6c[hi] monocytes enter into tissue, proliferate, and compete with proliferating resident cardiac macrophages to reestablish the steady-state macrophage pool. After repopulation is complete, autonomy between tissue macrophages and monocytes is restored. The pool of resident cardiac macrophages has undergone “ontological reprogramming” and is now primarily composed of adult-derived macrophages. In the absence of circulating blood monocytes (such as in Ccr2−/− mice), resident cardiac macrophages are fully capable of reestablishing steady-state levels solely through in situ proliferation.

(B) Alveolar macrophages. In the steady state, the majority of alveolar macrophages are derived from embryonic progenitors, and a smaller contribution is derived from HSCs. Alveolar macrophages are instructed by GM-CSF to differentiate into functional alveolar macrophages that prevent alveolar proteinosis. After depletion (CD163-DTR), numbers of resident macrophages are reduced, and repopulation occurs solely through in situ proliferation of resident lung macrophages. If resident macrophages receive significant genotoxic injury (lethal irradiation) as a depletion strategy, recruited monocytes gain a competitive advantage and replace resident, embryonically derived lung macrophages. If recruited monocytes cannot receive GM-CSF signals, then alveolar macrophages, despite genotoxic injury, are capable of repopulating the lung macrophage niche.
### Table 1

Tools Used for Defining Macrophage Lineages

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Result</th>
<th>Strength</th>
<th>Limitation</th>
<th>References</th>
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<tr>
<td>Runx1&lt;sup&gt;CreER&lt;/sup&gt;</td>
<td>identifies yolk-sac-derived macrophages</td>
<td>is expressed very early during development</td>
<td>has a very limited window; results in incomplete labeling during development</td>
<td>Hoeffel et al. (2012); Ginhoux et al. (2010)</td>
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<tr>
<td>Csf1r&lt;sup&gt;CreER&lt;/sup&gt;</td>
<td>identifies yolk-sac-derived macrophages</td>
<td>is expressed very early during development; has a longer window than Runx1&lt;sup&gt;CreER&lt;/sup&gt;</td>
<td>results in incomplete labeling during development</td>
<td>Epelman et al. (2014); Schulz et al. (2012)</td>
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<td>Flt3&lt;sup&gt;Cre&lt;/sup&gt;</td>
<td>defines cells that pass through definitive hematopoiesis (HSCs)</td>
<td>has strong reporter induction in circulating monocytes in adult animals; differentiates well between embryonic and adult macrophages; is excellent at following population dynamics during stress</td>
<td>does not differentiate well between embryonic lineages (yolk sac versus fetal monocytes); might not label some HSC-derived macrophages during times of rapid expansion (i.e., in early development or after adoptive transplant)</td>
<td>Epelman et al. (2014); Schulz et al. (2012); Hashimoto et al. (2013); Boyer et al. (2011)</td>
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<td>Cx3cr1&lt;sup&gt;GFP&lt;sup&gt;+&lt;/sup&gt;&lt;/sup&gt;</td>
<td>identifies cells currently expressing CX3CR1&lt;sup&gt;hi&lt;/sup&gt; macrophages</td>
<td>corresponds well to yolk-sac-derived F4/80&lt;sup&gt;hi&lt;/sup&gt;CD11b&lt;sup&gt;lo&lt;/sup&gt; macrophages during development only</td>
<td>is not reliable for differentiating lineages in the adult</td>
<td>Yona et al. (2013); Jung et al. (2000)</td>
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<td>Cx3cr1&lt;sup&gt;Cre&lt;/sup&gt;</td>
<td>labels all cells that pass through a CX3CR1&lt;sup&gt;+&lt;/sup&gt; stage</td>
<td>identifies traditionally CX3CR1&lt;sup&gt;+&lt;/sup&gt; populations as having passed through the CX3CR1&lt;sup&gt;+&lt;/sup&gt; stage</td>
<td>is less useful for differentiating between recruited and resident macrophages in the adult</td>
<td>Yona et al. (2013)</td>
</tr>
<tr>
<td>Cx3cr1&lt;sup&gt;CreER&lt;/sup&gt;</td>
<td>has the potential to label embryonic macrophages at different stages of development</td>
<td>identifies very strong reporter expression in tissue macrophages expressing high levels of CX3CR1; only transiently labels monocytes, which allows for differential targeting</td>
<td>is limited to tissue macrophages that express CX3CR1 at the time of induction</td>
<td>Yona et al. (2013); Goldmann et al. (2013); Parkhurst et al. (2013)</td>
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<td>Rag1&lt;sup&gt;Cre&lt;/sup&gt;</td>
<td>identifies lymphomyeloid progenitors</td>
<td>is the only technique for specifically labeling lymphomyeloid progenitors</td>
<td>it is unclear whether progeny persist into adulthood</td>
<td>Böiers et al. (2013)</td>
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<td>Parabiosis</td>
<td>demonstrates tissue-specific replacement of resident macrophages by chimeric monocytes</td>
<td>does not use irradiation; turnover is dependent on the steady state rather than tissue injury; use of Ccr2&lt;sup&gt;−/−&lt;/sup&gt; recipients increases chimerism to &gt;90%</td>
<td>typically demonstrates only 30% chimerism in wild-type mice; surgery might alter steady-state trafficking</td>
<td>Hashimoto et al. (2013); Epelman et al. (2014); Ajami et al. (2011); Ajami et al. (2007)</td>
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<td>Sublethal irradiation and bone marrow transplant</td>
<td>induces chimerism in blood monocytes without eradicating resident macrophages</td>
<td>demonstrates blood monocyte dependence of tissue macrophages over long periods of time; tissue shielding can minimize local radiation injury</td>
<td>radiation might alter steady dynamics (i.e., injured tissue recruits monocytes)</td>
<td>Epelman et al. (2014)</td>
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Table 2

Future Directions

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<th>Question</th>
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<td>Do tissue macrophages that live in mixed ontological lineages differ</td>
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<td>transcriptionally?</td>
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<td>Does targeting tissue macrophages on the basis of ontological</td>
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<td>lineage reveal differential functions?</td>
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<td>What is the extent of tissue macrophage plasticity?</td>
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<td>Why do tissue macrophages proliferate during inflammation?</td>
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