Resident and Monocyte-Derived Macrophages in Cardiovascular Disease

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Abstract: Macrophages are ubiquitous cells that reside in all major tissues. Counter to long-held beliefs, we now know that resident macrophages in many organs are seeded during embryonic development and self-renew independently from blood monocytes. Under inflammatory conditions, those tissue macrophages are joined and sometimes replaced by recruited monocyte-derived macrophages. Macrophage function in steady state and disease depends on not only their developmental origin but also the tissue environment. Here, we discuss the ontogeny, function, and interplay of tissue-resident and monocyte-derived macrophages in various organs contributing to the development and progression of cardiovascular disease. (Circ Res. 2018;122:113-127. DOI: 10.1161/CIRCRESAHA.117.311071.)

Key Words: cardiovascular disease ■ hematopoietic system ■ inflammation ■ macrophage ontogeny ■ tissue-resident macrophages

Over the last decade, sophisticated fate mapping, adoptive transfer, and parabiosis techniques enabled experiments that challenge the hierarchical mononuclear phagocyte system proposed by Van Furth et al1 in 1972. Immature cells in the bone marrow were thought to give rise to circulating monocytes that continuously migrated to peripheral tissues, where they differentiated into macrophages. We now know that many tissue macrophage populations arise during embryonic development, are seeded well before birth and are thereafter maintained by local proliferation rather than monocyte recruitment. Consequently, resident macrophages in many organs are completely or at least partly independent from blood monocytes generated by medullary or extramedullary hematopoiesis.2–4

Embryonic macrophage development occurs in several waves.5,6 In the first primitive hematopoiesis, myeloid progenitors arise in the yolk sac posterior plate mesoderm around embryonic day 7. A few days later, the yolk sac hemogenic endothelium gives rise to erythromyeloid precursors that can differentiate into fetal macrophages. As circulation establishes, erythromyeloid precursors subsequently migrate to the fetal liver where they differentiate into erythrocytes, megakaryocytes, granulocyte, mast cell, and monocytes. At the same time, the first hematopoietic progenitors arise in the aorta-gonad-mesonephros, the major vitelline and umbilical arteries and the placenta and seed the fetal liver around day 10. Here, definite hematopoietic stem cells (HSCs) differentiate and thus make the fetal liver the main site of embryonic hematopoiesis from embryonic day 11 onwards. Because tissue macrophage populations arise from different waves of macrophage development, we describe their ontogeny—if known—at the beginning of each section.

In the adult, new monocytes are generated by hematopoiesis, a hierarchical differentiation program emanating from HSCs. In the mouse, 2 main monocyte subsets can be identified by their Ly6C expression levels. Classical Ly6Chigh monocytes differentiate from Ly6C<sub>high</sub> progenitors, whereas the nonclassical or alternative Ly6C<sub>low</sub> monocytes transdifferentiate from Ly6C<sub>high</sub> monocytes in a process that depends on the transcription factor Nr4a1.7 Under healthy conditions, Ly6C<sub>low</sub> monocyte function closely associates with the vasculature, where they patrol along vessel walls and dispose of damaged endothelial cells.8,9 Classical Ly6C<sub>high</sub> monocytes are recruited to sites of inflammation, where they differentiate into macrophages. Ly6C<sub>high</sub> monocytes can also infiltrate peripheral tissues under homeostatic conditions and have been shown to transport antigens to draining lymph nodes.10 Human monocyte subsets can be identified by their expression of cluster of differentiation (CD)16 (Fc receptor) and CD14 (a coreceptor for lipopolysaccharides). The current working framework of monocyte subsets will likely become more complex, as emerging data suggest a continuum—rather than 2 or 3 defined states—between the subsets. The use of single cell RNA sequencing and mass cytometry in conjunction with high-dimensional antibody panels has improved subset identification resolution and thus allowed unbiased detection of previously unknown monocyte and macrophage phenotypes in different tissues under homeostatic conditions.11,12 Most of these phenotypes are still awaiting functional characterization, which is ultimately what makes a newly-described cell subset significant.

Both heterogeneous origins and distinct tissue microenvironments strongly shape resident macrophages’ expression profiles and functions. We see this clearly in splenic red pulp
macrophages, which express the heme-induced transcription factor Spi-C that allows them to manage large amounts of iron.13,14 Transplanting macrophages to a foreign environment can drastically change the gene expression profile and thus the function of the transplanted cells, thereby indicating that the tissue environment powerfully controls macrophage function. Transplanting peritoneal macrophages into the lung, for example, will induce a distinct alveolar macrophage expression profile in formerly peritoneal macrophages.15 Similarly, monocyte-derived macrophages can take over the functions of embryonic tissue macrophages if the latter are lost, for example, because of irradiation or after myocardial infarction (MI). Those newly-derived macrophages constantly renew, with a turnover rate of ≈ 5 weeks in steady state.16 Parabiosis experiments performed by both Epelman et al17 and Heidt et al16 reported tissue macrophage renewal in the healthy heart to be mostly independent from blood monocytes.

The homeostatic function of cardiac tissue macrophages remains somewhat unclear. Gene expression analysis suggests these macrophages, for example, play a role in angiogenesis and immune quiescence.22 However, we still lack functional in vivo data that clearly define the role of cardiac macrophages in those processes. During development, CCR2+ and CCR2− macrophage populations occupy different cardiac locations associated with distinct functions.24 CCR2+ cells with embryonic ontogeny reside predominantly in the myocardial wall. They are important for normal development and, more specifically, development of the coronary vasculature. In contrast, CCR2− macrophages, which are mostly found in the trabecular projections of the endocardium, are dispensable for cardiac development.

A recent study described macrophages in the heart’s conduction system, particularly the adult atrioventricular and sinus node.26 These macrophages form direct connections to cardiomyocytes via Cx43 (connexin 43) gap junctions and depolarize in synchrony with the cardiomyocytes to which they connect. Interestingly, both Cx43-deficiency and macrophage depletion by diphtheria toxin (DT) injection in Cd11b−/− mice significantly impairs atrioventricular conduction. Macrophages enter a source–sink relationship with conducting cells and influence their action potential shape and duration. Taken together with reports that monocyte-derived macrophages possess functional ion channels,27-29 these data invite exploring macrophages’ role in conduction disorders and whether cells recruited to the myocardium under inflammatory conditions and cardiovascular comorbidities.19 Aging is associated with clonal hematopoiesis and a marked myeloid bias that may similarly increase the risk of cardiovascular disease.20,21 In this article, we discuss the multiple roles and interplay between tissue macrophages and blood-derived monocytes/macrophages in organ systems relevant to cardiovascular disease. We also review macrophages that do not reside in the heart or vasculature to provide a wider context and highlight potential organ system interactions.

**Heart and Arteries**

Myeloid cells in the murine heart constitute 7% to 8% of non-cardiomyocytes.6,22 Murine cardiac macrophages are commonly classified by their surface expression of CCR2 (C-C chemokine receptor type 2) and MHC-II (major histocompatibility complex II). Most cardiac macrophages are MHC-IIlow CCR2− and are derived from yolk sac progenitors.23,24 The adult heart contains a separate MHC-IIhigh CCR2+ macrophage population with predominantly embryonic origin. As cardiac MHC-IIhigh CCR2+ macrophages increase after birth, they may arise from MHC-IIlow CCR2− cells.23 The third and smallest macrophage population, which is CCR2+, derives from definitive hematopoiesis, that is, from HSCs in the fetal liver.25,26 Resident macrophages in the adult heart retain expression of the fractalkine receptor CX3CR1.2,25 Cardiac tissue macrophages constantly renew, with a turnover rate of ≈ 5 weeks in steady state.16 Parabiosis experiments performed by both Epelman et al23 and Heidt et al16 reported tissue macrophage renewal in the healthy heart to be mostly independent from blood monocytes.
impact cardiac conduction. Considering that microglia are essential for synaptic pruning, it will be interesting to investigate if cardiac macrophages participate in the development of the cardiac conduction system.

Arterial macrophages likely originate from yolk sac-derived erythromyeloid precursors and 1 additional wave of blood-derived monocytes shortly after birth. Thereafter, arterial macrophages self-maintain both in steady state and after exposure to bacteria.39 Like cardiac macrophages, arterial macrophages retain continuous CX3CR1 expression, which likely plays a role in their continuous survival and self-maintenance.30

Although murine cardiac and arterial macrophages have been extensively studied, data on human cardiac macrophages, their phenotypes, subsets and function during homeostasis and in disease are still sparse. Emerging technologies like mass cytometry and subset-specific noninvasive clinical imaging approaches will hopefully aid in addressing those knowledge gaps.

Myocardial Infarction

The temporal dynamics of different leukocyte populations were analyzed by flow cytometry of single cell suspensions at different time points after surgically-induced myocardial infarction in mice.31 Neutrophils are among the first leukocytes to accumulate in ischemic cardiac tissue. Recently, Li et al32 proposed a role for resident CCR2+ macrophages in initial neutrophil extravasation using a model of cardiac ischemia reperfusion and syngeneic heart transplantation. In their study, chemoattractants CXCL (C-X-C motif chemokine ligand)2 and CXCL5 produced by CCR2+ resident macrophages contribute to transendothelial leukocyte migration into the ischemic area. Monocyte recruitment (Figure 1) may occur as early as 30 minutes after ischemia onset and depends on CCR2 signaling, predominantly via CCL2 (C-C chemokine ligand 2).31,33–35 Within a few days, the infiltrating Ly6Chigh cells, which have a life span of only 20 hours, begin to differentiate into reparative Ly6Clo cells.36–38 Those reparative macrophages can persist for several weeks and renew partially by local proliferation.36 Ly6Clo monocytes are also recruited during this reparative phase, albeit in much lower numbers and with unclear functional consequences.38 In addition to the bone marrow, the spleen acts as a reservoir for monocytes after MI. Adrenergic signaling stimulates bone marrow HSC egress, consecutive seeding in the spleen and extramedullary hematopoiesis.39,40 In the initial 24 hours post MI, the spleen releases its monocyte reservoir; consequently, as much as 40% of infiltrating Ly6Chigh monocytes in the infarct originate from the spleen.40 Embryonic cardiac macrophages die locally shortly after MI and thus disappear from ischemic heart tissue.38 In the chronic phase post MI, the majority of macrophages is again maintained by local proliferation, with a smaller contribution from circulating monocytes.41

In addition to pursuing phagocytosis and effectorcytosis, macrophages produce important mediators post MI that establish crosstalk with other cardiac cell types (Figure 2). TNF-α (tumor necrosis factor α), released by macrophages but also by cardiomyocytes and endothelial cells, induces cardiomyocyte hypertrophy and thereby increases the chance of heart failure post MI. In patients, high TNF-α levels predict impaired cardiac function and increased mortality.42 Of note, in a genetic heart failure model TNF-α also protects cardiomyocytes from apoptosis.43 Macrophages influence fibroblasts via TGF-β (transforming growth factor-β), that, together with additional mediators, induces their conversion to myofibroblasts. Myofibroblasts thereupon express α-smooth muscle actin and produce essential collagen.44 Additionally, secreted proteolytic enzymes like MMPs (matrix metalloproteinases) degrade extracellular matrix and contribute to tissue remodeling and scar formation. Macrophage-derived VEGF (vascular endothelial growth factor) acts on endothelial cells and stimulates angiogenesis.31

In total, monocytes/macrophages promote infarct healing, as macrophage depletion drastically impairs healing and worsens disease outcome.45–48 However, systemically increased monocyte/macrophage numbers, as in ApoE−/− (apolipoprotein E)-deficient or chemokine Decoy Receptor D6-deficient mice, can be detrimental to infarct healing and remodeling.47,48 Further, reducing the number of infiltrating monocytes, for example, by limiting B-cell mediated mobilization or therapeutically reducing recruitment via CCR2 blockage, decreases infarct size and supports post-MI recovery in animal models.49,50 We hypothesize that therapeutic approaches limiting the inflammatory monocyte supply to the heart can improve post-MI recovery in patients with high systemic inflammatory activity.

Post-MI Heart Failure

Although the initial fibrosis post MI is necessary to aid stable scar formation and prevent ventricular rupture, extensive fibrosis can result in pathological cardiac tissue remodeling and long-term heart failure.50,51 The failing heart contains increased number of macrophages that expand by both local proliferation and further recruitment.36,41 The recruited monocytes originate from medullary and extramedullary hematopoiesis and have a net detrimental effect.41,52 Consequently, splenectomy reduces inflammatory infiltrates and improves cardiac geometry and function.52 The expanded cardiac macrophage population plays an important role in the progression of heart failure by secreting factors that directly or indirectly stimulate continued fibrosis.53 TGF-β directly induces expression of profibrotic genes in myofibroblasts, whereas indirect factors like angiotensin-II, PDGF (platelet-derived growth factor), or endothelin-1, produced by macrophages, endothelial cells, and fibroblasts, further stimulate the release of TGF-β from myofibroblasts and cardiomyocytes.

Cardiac Regeneration

Certain fish and amphibians can regenerate cardiac tissue after injury throughout adult life.54 In zebrafish, for example, the heart is able to fully regenerate after resection of ≥20% of the left ventricle.55 The de novo generated tissue is produced by cardiomyocyte proliferation.56,57

In rodents, the neonatal heart can regenerate in response to multiple injuries, including partial surgical resection, myocardial infarction, and cryoinjury.58–60 This regenerative ability depends on the presence of macrophages and, more specifically, on expansion of the embryo-derived CCR2+ macrophage population.61,62 Inflammatory response and regenerative
potential are regulated via both parasympathetic and sympathetic nerve activity. Either pharmacological inhibition of cholinergic nerve activity or surgical left vagus nerve ablation impairs cardiac regeneration in neonatal mice. Likely influential factors are, on the one hand, direct cell cycle regulators like Ccnd2 and Cdk4 and, on the other hand, inflammatory genes that are downregulated after vagotomy. A study by White et al implies involvement of sympathetic innervation as chemical sympathectomy by 6-OHDA treatment similarly impairs regeneration.

The adult murine heart lacks this regenerative ability because of a reduced capacity of adult cardiomyocytes to re-enter the cell cycle. Cardiac regenerative capacity may be modified by sympathetic and parasympathetic nerve activity, proper vascular regeneration and in part also because of an inflammatory monocyte/macrophage response that changes in the first 2 weeks after birth. Although macrophage expansion in neonates results from CCR2− subset proliferation, this embryonic population is lost in adult hearts after cardiac injury and is instead replaced by CCR2+ blood monocytes. Consequently, limiting CCR2+ influx by administering a CCR2-inhibitor can preserve embryonic tissue macrophages in the adult heart and thus improve cardiac repair.

**Hypertension**

After angiotensin II–induced hypertension, the cardiac Ly6C+ monocyte/macrophage population expands by both blood monocyte recruitment and local tissue macrophage

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**Figure 1. Macrophages in cardiovascular disease.** During myocardial infarction (MI), atherosclerosis and stroke, monocytes are supplied by medullary and extramedullary hematopoiesis in bone marrow and spleen. Monocytes infiltrate diseased tissues, differentiate into macrophages and proliferate locally. In MI, resident cardiac macrophages are lost, whereas arterial macrophages in atherosclerosis persist. Cerebral microglia are activated after stroke and contribute to disease progression and healing. In obesity, macrophage accumulation in adipose tissue stems from local proliferation of resident and recruitment of monocyte-derived macrophages.

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**Figure 2. Macrophage mediators and crosstalk after myocardial infarction.** TNF-α (tumor necrosis factor-α) acts on cardiomyocytes and can induce hypertrophy and cell death. TGF-β (transforming growth factor-β) induces conversion of fibroblasts to myofibroblasts that produce collagen necessary for scar formation. Proteolytic enzymes like MMPs (matrix metalloproteinases) contribute to tissue remodeling, whereas VEGF (vascular endothelial growth factor) acts on endothelial cells and stimulates angiogenesis.
proliferation. Additionally, angiotensin II–induced hypertension results in pathogenic macrophage accumulation in the adventitia and perivascular adipose tissue (PVAT), mediated by CCR2. Reducing macrophage numbers by inhibiting CCR2 may provide a strategy to lessen vascular remodeling and, consequently, hypertension. At the same time, the inflammatory response might be influenced by CD11b+ Gr1+ myeloid-derived suppressor cells, as depleting this cell population was reported to elevate the inflammatory hypertensive response and increases organ damage. More precise cellular analysis is warranted to reveal which myeloid cells or subsets are protective in hypertension, as myeloid-derived suppressor cells are an overly broad term that even includes neutrophils. Overall, the role of tissue-resident and monocyte-derived macrophages in the genesis and complications of hypertension is incompletely understood.

**Atherosclerosis**

Macrophages are critically involved in all stages of atherosclerosis, a chronic inflammatory disorder, affecting large- and medium-sized arteries, that results in acute myocardial infarction and stroke. Atherosclerosis is characterized by the formation of atherosclerotic plaques, which are vessel wall accumulations of lipids and immune cells covered by a fibrous cap composed of collagen. Macrophage contribution to atherosclerotic lesion formation and progression is extensively described elsewhere. In short, lesions typically start out with subendothelial lipid deposition and leukocyte recruitment. Macrophages in the lesion take up high amounts of lipids and become large foam cells (Figure 1). Those fatty streak lesions subsequently progress to larger plaques containing a necrotic core and a fibrous cap of varying stability. Continuous plaque growth can lead to vessel lumen narrowing and surrounding tissue ischemia. Organ ischemia occurs if the fibrous cap ruptures and the necrotic core is exposed, resulting in thrombosis at the site of the plaque or, in case of detachment, a traveling embolus that obstructs blood flow into peripheral tissues. Plaque erosion is a third process that results in thrombus formation, vessel occlusion, and organ ischemia. Erosion is characterized by a loss of endothelial cells and thrombus formation in the absence of plaque rupture. In humans, eroding plaques contain high amounts of fibrotic tissue and smooth muscle cells and can often be found in regions of disturbed flow. Recently, Franck et al elicited superficial plaque erosion in mice by endothelial injury and subsequent local flow perturbation. As in human eroding plaques, blood flow mediation in this setup disturbs the endothelium and causes neutrophil accumulation that is mediated by arterial TLR2 (toll-like receptor 2) expression. 

Monocytes recruited to atherosclerotic lesions are Ly6Chigh cells that originate from both medullary and extra-medullary hematopoiesis. The major chemokine receptors mediating monocyte transmigration are CCR2, CX3CR1, and CCR5; and deleting all 3 drastically reduces atherosclerosis in ApoE−/− mice. It was recently reported that smooth muscle cell-derived cells in atherosclerotic lesions express several macrophage markers, including CD11b, F4/80, and Mac3. These markers likely arise because of a phenotype switch from smooth muscle cells to macrophages or foam cells. That such a switch in cell identity contributes substantially to the macrophage population in atherosclerotic lesions is difficult to reconcile with parabiosis data demonstrating that all plaque macrophages derive from circulating monocytes. Curiously, the smooth muscle cell-derived macrophage-like cells do not express the leukocyte marker CD45. We think the relative contribution of phenotype transition from smooth muscle cells to macrophage-like cells is currently unresolved (and possibly minor). We further posit that the pathophysiological importance of this putative lineage transition requires clarification.

Parabiosis studies have shown that the monocyte expansion mechanism in atherosclerosis depends on plaque characteristics and state. Although newly developed lesions mostly recruit monocytes from the blood, more advanced lesions rely primarily on local macrophage proliferation, with a smaller contribution from new monocyte recruitment. However, even the locally proliferating macrophages derive from cells recruited from the circulation. How exactly tissue-resident arterial macrophages, of embryonic or neonatal origin, that continuously proliferate in steady state interact with the monocyte population in the atherosclerotic lesion remains to be seen.

Even advanced atherosclerotic lesions can regress. In mice, this phenomenon has been investigated in a variety of settings. Infusion of the human ApoA-I variant improves cholesterol efflux and decreases foam cell formation and macrophage content in ApoE−/− mice. Likewise, introducing ApoA-I via adenoviral gene therapy causes fast regression of atherosclerosis. In the so-called Reversa mouse, physiological blood LDL (low-density lipoprotein) levels can be reestablished by Cre-mediated inhibition of liver lipoprotein production. Alternatively, ApoE−/− aortas have been transplanted into wild-type mice. In those settings of plaque regression, the number of plaque macrophages declines and the remaining cells assume reparative, less inflammatory phenotypes. Rahman et al found that, after aortic transplantation, the mostly reparative macrophages in regressing lesions originate from the circulation, thereby indicating that even in the context of plaque regression new monocytes infiltrate the arterial wall. Those newly recruited macrophages arise from Ly6CChih monocytes that infiltrate in a CCR2-dependent manner and subsequently differentiate into macrophages in a STAT6 (signal transducer and activators of transcription 6)–regulated differentiation program. The confounding setting of arterial transplantation, the surgically-induced inflammation and the sudden, steep decrease in LDL cholesterol will require careful clinical studies to evaluate if this scenario translates to patients with regressing atherosclerosis.

Although not directly targeting macrophages but rather one of their inflammatory products, the recent CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) confirmed that modifying inflammation in patients with atherosclerosis is a viable strategy. CANTOS investigated the effect of IL-1β (interleukin-1β) inhibition in patients with previous myocardial infarction and increased high-sensitivity C-reactive protein levels. The treatment reduced inflammatory blood parameters in a dose-dependent manner and lowered circulating leukocyte numbers. Notably, canakinumab treatment decreased the incidence of cardiovascular events as...
compared with placebo by 15%, thus emphasizing the immune system as a highly promising therapeutic intervention point in cardiovascular disease.

**Bone Marrow**

Distinct macrophage populations, with to date unresolved ontogeny, reside in specific tissue niches in the bone marrow. In steady-state conditions, these macrophages most likely self-renew; however, if they are depleted by irradiation, the lost cells can be replenished by bone marrow myelopoiesis.\(^{17}\) Macrophages can be found within the bone marrow HSC endosteal and perivascular niches. These niches are characterized by the presence of long-term HSCs as well as stromal cells, including perivascular Nestin\(^{+}\) mesenchymal stromal cells, osteoblasts, CXCL12-abundant reticular cells, endothelial cells, and the aforementioned macrophages.\(^{95,96}\) In addition, macrophages are located in erythroblast islands.

Bone marrow macrophages anchor hematopoietic stem and progenitor cells (HSPCs) in the hematopoietic niche. Consequently, macrophage depletion results in HSPC mobilization from the bone marrow and increased numbers of circulating HSPCs. This was observed after genetic macrophage depletion, clodronate-liposome depletion, and DT treatment of CD169\(^{+}\)TRT mice.\(^{95,96}\) Consistent with this framework, macrophage depletion downregulates the expression of HSC retention factors such as CXCL12, angiopoietin-1, Kit ligand, and VCAM-1 (vascular cell adhesion molecule 1) in the bone marrow and specifically in Nestin\(^{+}\) mesenchymal stromal cells.\(^{96}\) As those depletion strategies act systemically, rather than tissue-specifically, and macrophages have been reported to similarly anchor HSPCs in the spleen, the increase in circulating HSPCs may in part result from depletion in extramedullary tissues.\(^{97}\) Surprisingly, macrophage depletion may also result in accumulation of quiescent and proliferative HSCs in the bone marrow, which is inconsistent with the idea of macrophages acting as an anchor.\(^{96}\)

Bone marrow macrophages are also important for regulating granulopoiesis. Circulating neutrophils have very short lifetimes of only 13 hours and are thus constantly cleared and produced in large quantities.\(^{99}\) Elimination of aged neutrophils in the bone marrow follows a circadian rhythm and is mediated by macrophages that phagocytose the aged cells and in turn induce release of hematopoietic progenitor cells via liver X receptor signaling.\(^{100,101}\) Macrophages are thus an active part of a circadian feedback loop that maintains steady state neutrophil numbers.

**Bone Marrow in Cardiovascular Disease**

After myocardial ischemia, the hematopoietic system is activated, and HSC and HSPC proliferation in the bone marrow increases, as does their migration into the blood stream. The elevated proliferation rate is attributed to CCR2\(^{+}\) HSPCs and their progeny and depends on expression of the transcriptional corepressor myeloid translocation gene on chromosome 16 (Mtg16).\(^{102}\) Interestingly, after MI CCR2\(^{+}\) HSPCs also raise the expression of TLR2 and TLR4, which may sense danger-associated molecular patterns released from the damaged myocardium. Although the actual endogenous ligand signaling via TLRs post MI remains undefined, there are several factors that could mediate the communication between ischemic cardiac tissue and the bone marrow, including the danger-associated molecular patterns HMBG1 (high-mobility group box 1), alarmins S100A8/9, and IL-1β. If and how stromal cell-like resident macrophages contribute to danger-associated molecular pattern detection and TLR signaling in the bone marrow remains to be determined. HMBG1 increases in the circulation of patients with MI and inversely correlates with disease outcome and survival.\(^{103,104}\) HMBG1 is secreted by macrophages as a delayed mediator of inflammation and signals through RAGE (receptor for advanced glycation end products), TLR2, and TLR4.\(^{105}\) Injecting HMBG1 in mice enhances CCR2\(^{+}\) HSPC proliferation.\(^{102}\) Alarmins S100A8/9A, which similarly signal through RAGE, TLR2, and TLR4, also have higher concentrations in MI patient myocardium and serum.\(^{106-108}\) Further, elevated S100A8/A9 levels associate with higher blood leukocyte numbers.\(^{107}\) The inflammatory cytokine IL-1β similarly increases in MI patient circulation.\(^{109}\) After coronary ligation in mice, antibody-mediated inhibition of IL-1β suppresses HSPC proliferation in the bone marrow. In this case, not only direct signaling via the IL-1-1 receptor expressed by HSPCs but also indirect effects via bone marrow stromal cells, possibly including macrophages, are critical to the effect of IL-1β.\(^{110}\)

**Spleen**

The spleen is home to at least 4 distinct resident macrophage populations that all fulfill specific functions in their anatomical niches: marginal, metallophilic, red pulp and white pulp macrophages.

The marginal zone, surrounding the white pulp, contains both marginal and metallophilic macrophages with to date undefined ontogeny. Because it is difficult to distinguish between marginal and metallophilic macrophages, many of the described functions are attributed to both cell types. In general, both populations are responsible for filtering pathogens from the circulation and subsequently producing cytokines to stimulate immune responses. To identify pathogens, those macrophages express a variety of pattern recognition receptors, including SIGNR1.\(^{111}\) Resident macrophages regulate B-cell development, retention and function within the marginal zone\(^{112,113}\), relatedly, proper macrophage function depends on B-cells, as macrophages do not express SIGNR1 in the absence of B-cells.\(^{114}\)

Red pulp macrophages seed prenatally from the fetal liver and self-maintain independently from blood monocytes.\(^{2,17}\) Critical for HSPC retention in the spleen via adhesion molecule VCAM-1,\(^{97}\) red pulp macrophages are also key to regulating red blood cell homeostasis as they phagocytose aged erythrocytes retained on their way through the red pulp.\(^{115}\)

**Spleen in Cardiovascular Disease**

In addition to the macrophages described above, the splenic red pulp contains a population of monocytes found in subcapsular clusters.\(^{40}\) After myocardial ischemia, those monocytes undergo angiotensin II-dependent mobilization and migrate to the ischemic tissue. Indeed, a major portion of monocytes in the heart within the first few days after myocardial infarction originate from the spleen.\(^{38,40}\) Consequently, the splenic...
monocyte reservoir is rapidly exhausted and subsequently refilled by differentiation of splenic macrophage/dendritic cell precursors. Similar to acute myocardial ischemia, extra-medullary myelopoiesis and monocyte recruitment from the spleen have been reported in chronic ischemic heart failure, atherosclerosis, and stroke.\textsuperscript{38,52,116–118}

**Adipose Tissue**

Adipose tissue is generally divided into white adipose tissue (WAT), beige and brown adipose tissue. WAT and beige adipose tissue consist of specific adipocytes (white, beige and brown) and fulfill distinct tasks at certain locations in the body.\textsuperscript{119–126} One specific depot is the perivascular adipose tissue (PVAT), which can have characteristics of either white or brown adipose tissue depending on its location. Although WAT locates throughout the body in humans, brown adipose tissue is solely localized in the neck, above the clavicular and around the spine. PVAT surrounds middle- and large-sized blood vessels.

In nonobese animals, WAT contains a variety of immune cells, including T-cells (natural killer cells, Th2 cells, and regulatory cells), eosinophils, and macrophages. Adipose tissue macrophages are at least in part of embryonic origin and are maintained by local proliferation in addition to recruitment, which occurs, for example, in obesity.\textsuperscript{122–125} In steady state, the immune cells within WAT create an anti-inflammatory environment crucial to homeostatic metabolism.\textsuperscript{126} Resident macrophages’ role in regulating insulin sensitivity requires alternative polarization by activation of PPAR\textgamma (peroxisome proliferator-associated receptor gamma) and is mediated by STAT6.\textsuperscript{127–130} In a paracrine loop, Th2 cytokines, including IL-4 and IL-13, released by adipocytes and eosinophils act on tissue macrophages,\textsuperscript{127,131} Macrophage-specific PPAR\textgamma-deficiency results in insulin resistance and tissue inflammation.\textsuperscript{127} Complementarily, administering IL-4 protects mice from insulin resistance and obesity.\textsuperscript{130,132} In obese mice, the number of macrophages in WAT drastically increases, and the macrophage population switches from an alternative to a more classical inflammatory activation state.\textsuperscript{123,133} The resulting chronic low-grade inflammation is believed to play an essential role in metabolic disorders.\textsuperscript{126,134}

**Adipose Tissue in Cardiovascular Disease**

Obesity is a major risk factor of cardiovascular disease. More specifically, obesity can include systemic inflammation, hyperlipidemia, and inflammatory adipokine production, all of which can initiate atherosclerosis and are thus considered risk factors.\textsuperscript{135,136} PVAT has been particularly implicated in the development and progression of atherosclerosis. PVAT, like other adipose tissues, is considered an endocrine organ and produces several molecules that can act on vessels in a paracrine fashion.\textsuperscript{137} As in WAT, the number of immune cells in PVAT increases in obesity. Transplanting PVAT—but not WAT—into ApoE\textsuperscript{−/−} mice leads to increased formation of atherosclerotic plaques at the site of implantation, thereby suggesting inflamed PVAT has proatherosclerotic properties.\textsuperscript{138} On the other hand, brown adipose tissue characteristics of PVAT may protect from atherosclerosis. Lack of PVAT in ApoE\textsuperscript{−/−} mice with conditional knockout of peroxisome proliferator-activated receptor-c in vascular smooth muscle cells causes reduced prostacyclin production in response to cold, which in turn might stimulate endothelial dysfunction and promote atherosclerosis.\textsuperscript{139}

In human samples, PVAT close to atherosclerotic plaques contains high numbers of macrophages,\textsuperscript{140} specifically at the interface between adventitia and PVAT.\textsuperscript{141} The leukocyte infiltration likely results from increased expression of chemotactants, including IL-8 and MCP-1.\textsuperscript{141,142} Additionally, the volume of human pericoronary adipose tissue, covering the larger branches of the coronary arteries, increases with age. Independent of the volume of visceral adipose tissue, pericoronary adipose tissue correlates with coronary artery calcification, giving rise to the view that PVAT may locally promote atherogenesis.\textsuperscript{143,144}

**Brain**

The best characterized tissue-resident macrophages in the brain are parenchymal microglia, that originate prebirth, likely from RUNX1+ yolk sac-derived progenitors around embryonic day 7.25,34 Microglia are independent from blood monocytes as they self-renew in a process requiring CSF-1R (colony-stimulating factor-1 receptor) signaling. If they are depleted by irradiation, microglia are partly replenished by infiltrating blood monocytes,\textsuperscript{3,145,146} Likewise, depletion of microglia by intracerebroventricular administration of ganciclovir in mice expressing the herpes-simplex virus suicide gene thymidine kinase under the CD11b-promoter causes replenishment of microglia by monocyte-derived cells.\textsuperscript{147,148} On the contrary, partial depletion of microglia in tamoxifen and DT treated CX3CR1\textsuperscript{CreER}Rosa26DTR mice or by application of CSF-1R-inhibitor is followed by rapid proliferation of remaining microglia, rather than by recruitment of peripheral myeloid cells.\textsuperscript{149,150}

Under homeostatic conditions, microglia in the brain are ramified cells with a small stationary cell body. Their cell processes constantly scan the surrounding area and communicate with neighboring cells including neurons, astrocytes, and endothelial cells. During neuronal development, microglia are essential for the survival and differentiation of neurons as they produce different neurotrophic factors, including IGF-1 (insulin-like growth factor 1).151 Microglia further mediate controlled neuronal cell death by noninflammatory phagocytosis, synaptic pruning, and synapse function.\textsuperscript{152–155}

In addition to microglia, the brain hosts at least 3 more resident macrophage populations, all with distinct expression profiles. Despite their differences, parenchymal microglia and nonparenchymal macrophages in the brain are closely related, and all are distinct from peripheral macrophages in terms of expression profiles.\textsuperscript{156} Surface markers of brain macrophage populations are closely related, which poses a considerable hurdle to separating these populations. Nonparenchymal macrophages are best characterized by their localization in the brain and their closest neighbors. Perivascular macrophages can be found next to endothelial cells, between laminin-positive endothelial and glial basement membranes. Leptomeningeal and choroid plexus macrophages, as the names suggest, are found in the meningeal space and the choroid plexus, respectively.\textsuperscript{157} Perivascular and leptomeningeal macrophages are long-lived and self-renewing cells replenished only by blood monocytes.
Brain Macrophages in Cardiovascular Disease

After cerebral ischemia, microglia react to danger-associated molecular patterns released by dying cells and subsequently produce cytokines, chemokines and other signaling molecules that recruit peripheral immune cells to the brain. After microglia activation, peripheral blood monocytes, dendritic cells, and neutrophils start infiltrating the brain within 1 day and stay for several days post stroke. The temporal dynamics of immune cell infiltration as well as the role of recruited monocyte-derived macrophages in the progression of stroke are still discussed controversially and likely vary depending on the used animal model and experimental set up.

Preventing monocyte infiltration by depleting peripheral monocytes/macrophages before middle cerebral artery occlusion was reported to be beneficial. In those studies, clodronate liposomes or CCR2 knockout decreased microglial activation and brain atrophy and improved overall recovery, indicating a negative role for monocyte-derived macrophages in the progression of stroke. However, preventing monocyte influx by clodronate treatment 1 and 2 days after induction of stroke by middle cerebral artery occlusion or photothermolysis increased cerebral hemorrhage and severely impaired neurological recovery. Yet another study detected no therapeutic effects of either clodronate treatment or prevention of monocyte infiltration by anti-CCR2 antibody application starting 24 hours before induction of stroke by photothermolysis.

In contrast, studies selectively depleting microglia in the context of stroke have mostly reported detrimental effects. Microglia depletion in mice carrying the herpes-simplex virus suicide-gene thymidine kinase under the CD11b-promoter in the context of stroke caused larger infarcts with higher excitotoxicity and altered neuronal activity. Similarly detrimental, microglia depletion by CSF-1R-inhibitor treatment 3 weeks before middle cerebral artery occlusion increased the size of the ischemic area, resulted in infiltration of more myeloid cells and exacerbated the proinflammatory astrocyte response. These studies indicate that microglia, despite their potential inflammatory profile, might protect neurons from excitatory stress and death by limiting inflammation after ischemia. Overall, microglia and macrophage response to stroke is likely a dynamic process, similar to the one after MI, that thus depends on the stroke model, mouse strain, timing, response amplitude, and cell phenotype.

Little is known about other brain-resident macrophages in the context of cardiovascular disease. Perivascular macrophages may play a role in hypertension, specifically the neurologic symptoms and cognitive impairment associated with chronic hypertension. Depleting perivascular macrophages by injecting clodronate liposomes into the cerebral ventricles decreases oxidative stress and restores cognitive functions in angiotensin II–induced hypertension.

Tools for Studying Macrophages

Lineage Tracing

Lineage tracing has been used to investigate the developmental origins of tissue macrophages. For those studies, genetically-modified mice carrying a fluorescent reporter gene downstream of a loxP-flanked STOP cassette and under a ubiquitously active promoter (eg, Rosa-loxP-STOP-loxP-YFP mice) were crossed with inducible Cre-lines. A frequently used model is the tamoxifen-inducible CX3CR1CreER line. If crossed with the reporter, tamoxifen injection in pregnant mice activates fluorescence in all CX3CR1 expressing embryonic cells and their progeny. Consequently, if the induction is stopped before the onset of hematopoiesis, blood-derived monocytes will not express the reporter and can thus be distinguished from fluorescent tissue macrophages that arise early during embryonic development. In addition to CX3CR1CreER, CSF1RCreER, and RUNX1CreER have been used to label macrophages originating in the embryonic yolk sac1,3,2,170. All monocytes and macrophages that arise specifically from adult hematopoiesis can similarly be labeled, for example with an inducible FLT3CreER line. The limitations of lineage tracing experiments mirror the limitations of the respective Cre-line, that is, the specific activity and leakage in other cells. For instance, LysMCre, frequently used to delete genes in macrophages, monocytes, and neutrophils, expresses Cre in the interventricular septum during development. Hence, a targeted gene may be excised in septal cardiomyocytes also. Care must be taken to consider that Cre reports on the gene expression history and not necessarily on the precise lineage.

Depletion Strategies

Depleting macrophages and distinct subpopulations allows researchers to investigate cell function by analyzing the consequences of cell absence. Clodronate liposomes, which are devoured by macrophages and induce cell death, are perhaps the easiest way to deplete macrophages and have been used for many years. Using this approach, however, it is nearly impossible to selectively target specific macrophage populations, as the systemic clodronate injection eliminates macrophages regardless of origin throughout the body. An exception might be locally injecting clodronate liposomes into the cerebral ventricles, an approach that has been applied to specifically eliminate perivascular macrophages in the brain. The technique’s limitations, including limited efficiency in some tissues such as the steady state myocardium, can be at least partly overcome by applying inducible genetic depletion strategies. In genetically-modified mice that express the DT receptor (DTR) under a macrophage-specific promoter, DT injection results in death of the targeted cell population. For example, CD11bDTR mice have been used to study the effects of cardiac macrophage depletion and CD166DTR mice to deplete bone marrow macrophages. Although this technique has a higher specificity than clodronate injection, DTR knockin mice are limited by the specificity of the chosen genetic marker. As only very few markers are exclusive for distinct macrophage populations, DT injection in most studies depletes mixed macrophage populations in many organs. An organ-specific macrophage depletion strategy is sorely needed and would be extremely useful for studying distinct tissue macrophage functions.
Therapeutic Targeting

Reducing the blood-derived monocyte infiltration to limit inflammation or atherosclerotic plaque progression has proven beneficial in mice after myocardial infarction and atherosclerosis. Preclinical models showed promising results by targeting different adhesion molecules, including intercellular adhesion molecules, VCAMs, selectins, and CCR2. 59,176,177 In addition to recruitment, studies have also targeted macrophages’ inflammatory status. Silencing transcription factor IRF5 (interferon regulatory factor 5) in cardiac macrophages post MI generated encouraging outcomes.37 Nanoparticle-based delivery of siRNA targeting IRF5 reduced inflammatory macrophage markers in the infarct, supported infarct healing and prevented heart failure. In atherosclerotic plaques, inflammatory status and macrophage proliferation have successfully been modified by targeted administration of statins incorporated into nanoparticles.176,177 Similarly, local anti-inflammatory treatment with glucocorticoids incorporated into small liposomes that are taken up by plaque macrophages showed positive effects on plaque size in a rabbit atherosclerosis model.178 To date these preclinical results have not been successfully translated into the clinic, as prednisolone-liposomes accumulated in human atherosclerotic plaque macrophages but did not elicit any significant anti-inflammatory effects.179

Monitoring

Monitoring macrophages is necessary to track the success of macrophage-targeting therapies. In addition, macrophage monitoring answers fundamental questions about the development, location, and spatio-temporal dynamics of macrophage populations in steady state and disease. Intravital microscopy is a powerful tool for distinguishing and following individual cells in vivo. Using fluorescent reporter lines, individual macrophages can be imaged over time to follow their interactions with other cells. The CX3CR1GFP mouse has been used to visualize CX3C3+ macrophages by intravital microscopy in the heart, spleen, and atherosclerotic plaques.40,102,180,181 Alternatively, cells can be labeled by injecting fluorophore-labeled antibodies, as is routinely done to visualize different cell types in the bone marrow.182,183 Macrophages’ high phagocytic activity also allows macrophage imaging with fluorescently-labeled nanoparticles.180

In human atherosclerotic patients, nuclear imaging can be used to image atherosclerosis using a variety of radiotracers targeting TSPO (translocator protein).193,194 In the brain, activated microglia have been monitored using radiotracers targeting TSPO (translocator protein).193,194 One of the PET tracers targeting TSPO is 18F-DPA-714, which has been used to image neuroinflammation in mice with cerebral ischemia, with signals peaking 2 weeks post stroke.195 Although there is very low TSPO expression in the healthy brain, TSPO is expressed not only by activated microglia but also by astrocytes and infiltrated peripheral immune cells in a variety of neurological disorders.196 Therefore, specific noninvasive monitoring of brain microglia remains a challenge.

Conclusions and Future Directions

This is an exciting time: increased availability of avant-garde technologies transforms research and accelerates knowledge gain that hopefully will lead to new cures soon. Enabled by interdisciplinary collaborations, we begin to understand the interactions among the innate immune, hematopoietic, and cardiovascular systems. Of particular interest is myelopoiesis, that is, the production of monocytes, which can give rise to inflammatory disease-promoting macrophages. We study how cardiovascular risk factors and comorbidities shape hematopoiesis, and these insights will become a springboard for therapeutic development. New tools now allow relatively easy genetic manipulation of the hematopoietic system. CRISPR/Cas9 mediated gene editing to modify human and murine HSPCs creates new opportunities for basic research and, in the near future, cardiovascular therapeutic studies.197–200 Single cell sequencing and mass cytometry provide unbiased discovery tools that can be applied to discover many new cell subsets, all of which will have to be tested for functional diversity. Intravital microscopy and noninvasive macrophage imaging are on the brink of broader adoption in basic and clinical research, respectively. In general, a more nuanced, well-resolved, and higher definition understanding of macrophage functions will enable targeting of disease-promoting cellular functions, whereas sparing macrophage activities or subsets that are essential for defending homeostasis. The results of the first successful large-scale clinical study on IL-1β neutralization are encouraging and hopefully only the beginning of a new era in cardiovascular immunotherapy.92

labeled with radioisotopes and fluorophores, thus allowing noninvasive PET imaging and its validation on a cellular level using the same particle.180,188 Macrophages have also been targeted more specifically, for example, via radioactively-labeled small molecules or nanoparticles binding to CCR2 and CCR5, which have been successfully applied to visualize plaque macrophages.199,200 We do however still lack a clinical imaging probe that offers high prognostic value by reliably distinguishing among different plaque characteristics (ie, stable, rupture-prone, regressing).

Overall, future advances will need to focus on developing imaging probes that monitor of macrophage subsets as well as dynamic changes in cell phenotype. Although there have been attempts to develop probes that specifically bind to proinflammatory macrophages in atherosclerotic lesions (eg, 68Ga-DOTATATE binding to somatostatin receptor 2), the specificity of those probes needs further validation.191,192

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References


64. Franch MF, Wason S, Asaas G, et al. Flow perturbation mediates neutrophil recruitment and potentiates endothelial injury via TLR2 in mice:


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