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Regulation and consequences of monocytois

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Summary

Monocytes are part of the vertebrate innate immune system. Blood monocytes are produced by bone marrow and splenic progenitors that derive from hematopoietic stem cells (HSCs). In cardiovascular disease, such as atherosclerosis and myocardial infarction, HSCs proliferate at higher levels that in turn increase production of hematopoietic cells, including monocytes. Once produced in hematopoietic niches, monocytes intravasate blood vessels, circulate, and migrate to sites of inflammation. Monocyte recruitment to atherosclerotic plaque and the ischemic heart depends on various chemokines, such as CCL2, CX₃CL1, and CCL5. Once in tissue, monocytes can differentiate into macrophages and dendritic cells. Macrophages are end-effector cells that regulate the steady state and tissue healing, but they can also promote disease. At sites of inflammation, monocytes and macrophages produce inflammatory cytokines, which can exacerbate disease progression. Macrophages can also phagocytose tissue debris and produce pro-healing cytokines. Additionally, macrophages are antigen-presenting cells and can prime T cells. The tissue environment, including cytokines and types of inflammation, instructs macrophage specialization. Understanding monocytois and its consequences in disease will reveal new therapeutic opportunities without compromising steady state functions.

Keywords

monocytes/macrophages; stem cells; spleen

Introduction

Monocytes are an essential part of vertebrates' cellular innate immune system. In humans and mice, steady state monocyte levels in blood are $2 \times 10^5/\text{ml}$. In mice, there are two major monocyte subsets, Ly-6c^{high} CX₃cr1^{low} and Ly-6c^{low} CX₃cr1^{high}, with proinflammatory function (1) and patrolling behavior (2), respectively. At least three human monocyte subsets have been identified(3): CD14⁺⁺ CD16⁻ (classical), which resemble Ly-6c^{high} monocytes; CD14⁺⁺ CD16⁺ (intermediate), with pro-inflammatory roles and CD14⁺ CD16⁺⁺ monocytes with patrolling behavior. All blood monocytes are produced by hematopoietic progenitors in the bone marrow and spleen. Monocyte release from the bone marrow follows circadian rhythms, and migration into and through blood is finely tuned by a

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panoply of signals. Once in blood, monocytes travel to inflammatory sites to aid in the host's initial immune response through, for instance, their role in mediating antimicrobial defense. In contrast to their numerous beneficial functions, monocytes also fuel acute and chronic inflammation in all major diseases. Our review focuses on monocytes in cardiovascular disorders, such as atherosclerosis and myocardial infarction, while highlighting instructive parallels and differences in other organ systems and diseases.

Once recruited into tissue, monocytes may differentiate into macrophages and certain dendritic cells. Major macrophage functions include phagocytosis of tissue debris and foreign materials, steady state defense and tissue repair after injuries. When macrophages become dysfunctional, they may promote disease; for example, ineffective clearance of dead cells by macrophages exacerbates atherosclerosis. The cells are also nodes of information transfer and signal amplification, facilitated, for instance, by amplifying pro- or anti-inflammatory cytokines. In addition, macrophages are antigen presenting cells and express major histocompatibility antigens.

Macrophage diversity stems from the tissue environment, including specific cytokines reflecting various inflammatory phenotypes, that instructs a wide range of macrophage specialization resulting in a plethora of macrophage subsets with distinct functions (4). Classically activated macrophages (M1) mediate inflammation and anti-microbial immunity. Alternatively activated macrophages (M2) are involved in wound healing and immunity against parasites. In addition to these, IL-10-producing regulatory macrophages and tumor-associated macrophages (TAMs) have been reported. Although TAMs have been thought similar to M2 macrophages, a recent study showed they are actually more like M1 macrophages (5). It has become clear that understanding functionally distinct monocyte and macrophage subsets will lead to advanced, narrowly targeted therapeutics that modulate the presence or character of harmful myeloid cells while avoiding severe side effects that typically arise from compromising important monocyte and macrophage steady-state functions.

Macrophage origin: blood monocytes versus tissue residents

Myeloid cells, including monocytes, arise from two successive versions of hematopoiesis, 'primitive' and 'definitive' hematopoiesis, that occur during development (6). During primitive hematopoiesis in the embryonic stage, red blood cells produced by the yolk sac address the embryo's increased oxygen demands during rapid growth. The yolk sac also gives rise to primitive tissue macrophages that can self-maintain in tissue by proliferation (7). For example, brain microglia renewal is independent of circulating progenitors, i.e. circulating bone marrow derived monocytes, and microglia are sustained by proliferation throughout adult life (8). Using mice with constitutive or conditional Cre recombinase in CX₃CR1 loci, Steffen Jung and his colleagues (9) demonstrated that tissue resident macrophages, including peritoneal, splenic and lung macrophages, plus Kupffer cells, are derived from the yolk sac before birth. During steady state, these cells are maintained independently from monocyte input. In an interesting contrast, steady state IL-10-producing CX₃CR1^{high} resident intestinal macrophages derive from Ly-6c^{high} monocytes (10, 11). Intestinal inflammation leads to accumulation of TLR-responsive CX₃CR1^{int} macrophages

that produce inflammatory cytokines. Nevertheless, intestinal resident macrophages as well as inflammatory macrophages in the gut derive from the same precursor.

In the heart, monocytes and macrophages are crucial to healing after ischemic injury of the myocardium (12-14). They clear dead cells after infarction and produce cytokines that facilitate tissue repair. Genetic fate mapping revealed that steady state cardiac macrophages are derived from the yolk sac and fetal monocyte progenitors (15). They self-maintain by local proliferation in the myocardium (16). However, in inflammation induced after myocardial infarction, the resident macrophage pool is replaced by macrophages that derive from Ly-6c^{high} monocytes (16). Likely depending on the changing environmental cues, Ly-6c^{high} monocytes may give rise to inflammatory macrophages early after ischemic injury but also to macrophages with pro-resolution phenotypes if recruited later (13). The origin of macrophages and dendritic cells that reside in blood vessel walls during steady state has yet to be described. Given the data recently obtained from many organ systems including the heart, these cells likely self-maintain locally, independent from blood monocytes, despite being so close to this pool of cells.

Role of downstream myeloid progenitors in monocyte production

The very short turnover rate for monocytes and macrophages in inflammatory tissues (17) indicates a sufficiently increased myeloid cell supply, likely through continuous replenishment by hematopoietic progenitor cells (Fig. 1). Macrophage dendritic cell progenitors (MDPs) can give rise to monocytes (18) and dendritic cells but not granulocytes (19). Interestingly, this finding was challenged by a recent study (20) that demonstrated that macrophage and dendritic cells do not share a common progenitor. Granulocyte-macrophage progenitors (GMPs), precursors of MDPs, can give rise to granulocytes. MDPs express CX₃CR1, which distinguishes them from GMPs, which do not express the receptor. CX₃CR1-deficient mice display normal monocyte levels in the bone marrow; however, MDPs isolated from CX₃CR1-deficient mice produce fewer monocytes in recipients' spleens after adoptive transfer when compared to monocytes isolated from wild type mice, indicating that CX₃CR1 is important in the development, recruitment, proliferation, and survival of monocytes in the spleen (19). MDPs also express CD115, the receptor for csf-1 (also known as the M-CSF receptor), which promotes survival, proliferation, and differentiation of macrophages. In addition to giving rise to monocytes, MDPs can also differentiate into common dendritic cell precursors (CDPs). MDPs and CDPs are phenotypically overlapping cell populations, though CDPs lack the ability to differentiate into monocytes and can only produce dendritic cells. A committed monocyte and macrophage progenitor that differentiates into monocytes but not dendritic cells has recently been identified in the bone marrow (21).

Role of upstream HSCs in monocyte production

Downstream myeloid progenitors were long thought to be the immediate source of myeloid blood cells during acute or chronic inflammation, but MDPs and GMPs have limited lifespans and cannot self-renew. Hence, they must be continuously replenished by upstream self-renewing hematopoietic cells. We now know that hematopoietic stem cells (HSCs) are

the source of myeloid blood cells. HSC frequency in the bone marrow is 1 in 10,000 cells, but only 1 in 100,000 cells in the blood. A major difficulty in HSC research has been identifying and characterizing the cells *in vivo*, since they behave like other hematopoietic cells in *ex vivo* culture. The 'gold standard' for identifying HSCs is their ability to reconstitute all lineages in lethally irradiated mice. In steady state, the vast majority of HSCs are quiescent, as only ~5% (or less) are in the cell cycle (22), and HSCs divide every 145 days or about 5 times in a mouse's life time (22). During stress, however, these stem cells enter the cell cycle to produce hematopoietic progenitor cells (23). In an *in vivo* mouse model of *Mycobacterium avium* infection, HSC proliferate in response to interferon- γ (IFN γ) (24). HSCs sorted from IFN γ -deficient mice have lower proliferation in steady state and inflammation. In response to interferon- α (IFN α) treatment, dormant HSCs exit G₀ phase and enter the cell cycle (25). IFN α -triggered HSC proliferation is STAT1 and Sca-1-dependent. IFN-mediated HSC proliferation is at least partly dependent on IFN receptor expression by HSCs (26). Most recently, we found that psychosocial stress in mice increases SLAMF6 HSC numbers in the bone marrow and instigates higher proliferation rates (27). Despite these changes, functional long-term HSC numbers, quantified by limiting dilution assays, remained constant. This illustrates that SLAMF6 surface markers are not specific for long-term HSC and that this most upstream gate contains a heterogeneous stem cell population.

A recent study (28) demonstrated that short-term HSCs produce copious cytokines in response to Toll-like receptor (TLR) signaling. Among these HSC-derived cytokines, IL-6 acts to instigate downstream progenitors' myeloid differentiation in a paracrine manner. The study also showed that HSC have functional TLR/ NF- κ B signaling capacity that can be directly activated by TLR-4 and TLR-2 ligands. Cytokines produced by HSC transferred by bone marrow transplantation may have regulatory roles in hematopoietic cell reconstitution. From these studies (24, 25, 28), it is clear that circulating alarmins liberated during infection or injury can push quiescent HSC into the cell cycle. However, we do not fully understand precisely how HSCs can sense danger. Especially in the setting of cardiovascular disease, data on how HSCs are alerted are scarce. There may be two scenarios, either (i) direct sensing through pathogen-associated molecular pattern (PAMP) receptors expressed by HSCs or (ii) indirect sensing through cells that regulate HSC activity in the bone marrow microenvironment. To directly sense danger and an increased need for peripheral leukocytes to defend the host, HSCs express PAMPs. Indeed, HSCs express TLRs and their co-receptors (29). Stimulating HSCs with TLR ligands propels HSC into proliferation (30) and leads to GMP differentiation.

Increased HSC proliferation in response to infection or injury may also be triggered by changes in bone marrow microenvironment. For example, myocardial infarction decreases HSPC retention factors such as Cxcl12, Scf, Ang-1, and Vcam-1 (31). This drastic change may increase HSC proliferation and release cells from their niches. An ensemble of supporting cells that reside in hematopoietic tissue in close proximity to HSC and hematopoietic progenitors, including macrophages (32, 33), regulatory T cells (34), endothelial cells (35, 36) and CXCL12-abundant reticular cells (37) maintain the hematopoietic niche and regulate HSC proliferation and differentiation. Changes in niche

cell activity during acute or chronic inflammation influence hematopoiesis (Fig. 1). For example, high levels of G-CSF produced in atherosclerosis may suppress CXCL12 production by osteoblasts, leading to HSC proliferation and release. LPS treatment, which mimics systemic infection, increases MCP-1 production by bone marrow endothelial cells and nestin⁺ mesenchymal stem cells, which results in monocyte release into the blood (38). Another example is the activation of the parathyroid hormone receptor, which induces expression of IL-6, RANKL and Jagged1 in osteoblasts and thus influences hematopoiesis (39, 40).

We know relatively little about the pathways HSCs use to sustain myelopoiesis during inflammation. There are several transcription factors reported to be involved in myeloid lineage determination (Table 1), including PU.1, which binds to GATA-binding protein 1 to inhibit commitment towards megakaryocyte-erythroid progenitors, thus facilitating myeloid differentiation (41). Another important transcription factor, the CCAAT/enhancer binding protein (Cebpa), directs GMP differentiation. Interestingly, committed B and T lymphocytes can be transdifferentiated into functional macrophages through Cebpa expression (6, 42, 43). The early growth response gene-1 and IFN consensus sequence binding protein (Irf8) can selectively induce monocyte and macrophage differentiation (44-47). In contrast, Gata-1 complements PU.1 and inhibits myeloid differentiation (48). Although long-term HSC are pluripotent and give rise to all blood cells, myeloid-biased HSC, such as PU.1⁺ M-CSFR⁺ HSC (49) and MyrP HSC (50) are poised to react to danger by giving rise to myeloid cells important for host defense.

Monocytosis in cardiovascular diseases

Myocardial infarction (MI) triggers monocyte recruitment to the infarct in two sequential phases (14). At first, Ly-6c^{high} monocytes are recruited via MCP-1, which is followed by Ly-6c^{low} monocyte recruitment via fractalkine. It is clear that monocyte recruitment is essential for infarct healing; however, the precise tasks and fates of involved cells are under intense investigation. Ly-6c^{high} monocytes may differentiate into macrophages to clear myocardial resident cells that died of ischemia, and Ly-6c^{low} monocytes may facilitate the healing process. During the first week after myocardial ischemia, monocyte and macrophage turnover in the infarct is surprisingly quick (<24 hours) (17). This shows that reactive leukocytosis after myocardial infarction, which occurs in mice and humans and correlates closely with survival, plays an important role in the just-in-time leukocyte supply to the infarct. Depleting monocytes after MI leads to a precipitous drop in monocyte and macrophage numbers in both the blood pool and the infarct, indicating that blood supply and cell levels in the inflamed end-organ tissue are closely linked. Oversupply of blood monocytes in mice with pre-existing atherosclerosis increases recruitment into the ischemic heart and impairs healing (51). These observations motivated us to study cell supply as a therapeutic target, hypothesizing that decreasing cells that can migrate into the infarct will reduce inflammatory macrophage numbers in the heart and enhance resolution of inflammation. Indeed, reducing the expression of the chemokine receptor CCR2 by nanoparticle-enabled RNAi attenuates monocyte traffic to the heart (52) and reduces post-MI heart failure (53).

Immediately after MI, splenic monocytes deploy to the infarct (54) and drastically, though temporarily, reduce spleen size in mice after coronary artery ligation. Soon thereafter, MI-induced extramedullary hematopoiesis replenishes monocytes in the spleen, which continues IL-1 β -dependent monocyte supply to the infarct (17). In the absence of the orphan nuclear hormone receptor, Nr4a1, Ly-6c^{high} monocytes express higher CCR2 levels (13), increasing inflammatory monocyte accumulation in the infarct. Moreover, Nr4a1-deficient Ly-6c^{high} monocytes differentiate into abnormally inflammatory macrophages that resemble M1 macrophages and lead to ventricular dysfunction after MI. M1 macrophages can promote inflammation and destruction of extracellular matrix, whereas M2 macrophages facilitate extracellular matrix regeneration and angiogenesis. Interferon regulatory factor 5 (IRF5) inhibition makes macrophages M2-biased and hastens myocardial healing after MI (55).

Atherosclerosis is characterized by pathological lipid deposition in the arterial wall. The lipid-rich atherosclerotic plaques are infiltrated by inflammatory cells, including monocytes. Atherosclerosis induces profound expansion of Ly-6c^{high} monocytes into the blood (56, 57). In mice with atherosclerosis, bone marrow hematopoietic stem and progenitor cells (HSPC) relocate to the spleen. The cells proliferate and differentiate into Ly-6c^{high} monocytes in presence of GM-CSF and IL-3 in the splenic red pulp (58). These inflammatory monocytes intravasate, circulate in the blood and finally accumulate in atheromata. The recruited monocytes then differentiate into macrophages, ingest lipids and turn into foam cells. During the first 4-8 weeks after initiation of a high fat diet, plaque macrophages are maintained by monocyte recruitment from the blood. In older mice, scavenger receptor A-mediated local proliferation of macrophages dominates macrophage supply to plaque (59). Even in these later stages, plaque macrophages derive from bone marrow-produced monocytes. Interestingly, the migrating intermediate in blood, i.e. the monocyte, is temporarily unable to proliferate. Once it differentiates into a macrophage, the cell regains the ability to divide, like its bone marrow progenitors. The processes that inhibit monocyte proliferation, and regulate the switch from recruiting to locally proliferating macrophage supply in plaque, are mostly unknown. Studies exploring these cellular mechanisms may reveal new therapeutic targets that support resolution of inflammation.

Though the mechanisms of monocyte activity during atherosclerosis are still being investigated, researchers have made progress on its causes. Work by Allan Tall's group (60-62) showed that defective cholesterol efflux increases HSC proliferation, and accelerates myelopoiesis and ultimately fuels inflammatory macrophage content in atherosclerotic plaque. ApoE regulates HSPC proliferation through cholesterol efflux-promoting ABC transporters ABCA1 and ABCG1. In ApoE-deficient mice fed a high fat diet, this regulation is disturbed, resulting in higher HSPC proliferation in the bone marrow and spleen. This leads to systemically increased myelopoiesis. Moreover, splenic macrophages and dendritic cells in mice that lack ABCA1 and ABCG1 produce high levels of IL-23, which results in high serum G-CSF levels (63) that favor lineage decisions toward granulocytes rather than macrophages.

Recurrent myocardial infarction occurs frequently and is often fatal. To better understand the mechanism of recurrent MI, we investigated atherosclerosis progression in ApoE^{-/-} mice after coronary ligation. We found that mice had larger plaques 3 weeks after ischemic

injury of the heart or brain. Moreover, the plaques had higher cathepsin protease activity, as measured by fluorescence molecular tomography-computed tomography and fluorescence reflectance imaging, indicating increased lesion 'vulnerability'. Quantitative PCR of aortic roots revealed elevated levels of inflammatory cytokines, such as IL-6, MMP9, MPO and Ly-6c, consistent with higher numbers of myeloid cells and monocytes in the aorta. MI activated the sympathetic nervous system, which suppressed CXCL12, SCF, Ang-1 and VCAM-1 production in bone marrow niche cells and triggered HSPC release from the bone marrow into the blood stream. The egressed HSPC accumulated in the spleen and proliferated in a stem cell factor (SCF)-dependent manner, leading to systemic monocytosis. Inflammatory monocytes recruited to atherosclerotic plaques enriched lesions' protease, decreased fibrous cap thickness, and increased necrotic core size. Our findings in ApoE^{-/-} mice (31) accord with recently published human data. Chen et al. assessed non-culprit coronary lesion progression in patients with ST elevation myocardial infarction (STEMI) using three-dimensional quantitative coronary angiography over a mean follow-up period of 12.3 months (64). Compared to a coronary artery disease control group, STEMI significantly accelerated atherosclerosis in non-culprit coronary lesions. A different study by Kim et al. (65) revealed significantly higher uptake of the PET imaging agent ¹⁸F-FDG in the spleen and bone marrow of patients with acute myocardial infarction. This clinical molecular imaging strategy reports on tissue-level glucose uptake. While not specific to a certain cell class, the increased imaging signal can be linked to the higher energy demands of cycling stem cells that need to increase protein production when entering the cell cycle (66). Overall, these clinical data indicate increased cell proliferation after acute ischemic events in the hematopoietic organs. Signer *et al.* (66) also revealed a positive correlation between spleen and bone marrow PET signal and higher PET imaging signal in carotid arterial plaques. This correlation suggests that inflammatory activity in human atherosclerotic plaque is not an isolated process, but rather intricately coupled with systemic monocyte levels and the activity of their progenitors in the spleen and bone marrow.

Release of monocytes and their progenitors from hematopoietic organs

Though it is a rare event, HSC do egress from bone marrow and circulate in blood during steady state. The exact purpose of HSC traveling through extramedullary tissue is unclear, though circulating HSC are known to conduct surveillance, and we do know HSC migration exhibits robust circadian fluctuation that peaks at Zeitgeber time (ZT) 5 (67). HSC release from the bone marrow niche depends on the sympathetic nervous system (SNS), which, when activated, decreases levels of HSC retention factors, such as CXCL12 that are produced by bone marrow niche cells, resulting in HSC release into the blood stream. At night, the sympathetic nervous system mediates increased expression of adhesion molecules and triggers HSC homing back to the bone marrow (68). These observations are supported by the finding that HSC injected into lethally irradiated mice at night engraft better than if this transfer is done during the day.

Like HSC, Ly-6c^{high} inflammatory monocytes also egress with diurnal variations, peaking high at ZT 4 and reaching nadir at ZT 16 in the blood and spleen (69). The amplitude of this normal circadian rhythm is quite astounding, as monocyte blood levels can easily be seen to double depending on the time of blood sampling. These cellular changes are accompanied

by oscillating expression of clock genes- *Bmal1*, *Nr1d1* and *Dbp* in monocytes. If mice were infected with *Listeria monocytogenes* when blood monocyte numbers were high, higher mortality occurred. This high mortality was attributed not to high systemic bacterial burden but rather to a massive ‘cytokine storm’ resulting in septic shock. Ly-6c^{high} monocyte fluctuation depended on *Bmal1*-mediated regulation of *Ccl2* levels.

Besides circadian cycling, monocytes leave the bone marrow during both infection and sterile inflammation, such as myocardial infarction and atherosclerosis. Monocytes were shown departing the bone marrow after an LPS challenge that correlated with high MCP-1 levels not in the blood but in mesenchymal stem cells and CXCL12-abundant reticular cells that line bone marrow sinusoids (38). Conditionally deleting *Mcp-1* in these two bone marrow cell types abrogated Ly-6c^{high} monocyte release from the bone marrow. Our group observed that the spleen acts as a monocyte reservoir after myocardial infarction (70). Monocytes are stored in subcapsular red pulp in the spleen. Upon acute injury, such as myocardial infarction, angiotensin II- angiotensin 1 receptor signaling promotes splenic monocytic motility and emigration from the spleen (Fig. 1). These monocytes are then recruited to the site of inflammation. Thus the spleen is a reservoir for rapid monocyte deployment that can regulate inflammation after acute injury by, for instance, inhibiting systemic angiotensin-2 signaling. Using an angiotensin converting enzyme inhibitor, a drug that is clinically used for treatment of hypertension, we were able to dampen splenic monocyte release and decrease their migration to acute infarcts in mice (71).

Recruitment of monocytes to sites of inflammation

Monocytes are recruited from the blood to sites of inflammation where they can either directly take part in inflammation or differentiate into macrophages that mediate inflammation. Monocytes are also recruited to infected tissues, as reviewed in detail elsewhere (72). During infection, monocytes may also differentiate into dendritic cells, resulting in anti-microbial inflammation. Additionally, these monocyte-derived dendritic cells can prime cytotoxic T cells. Although dendritic cells’ ability to stimulate adaptive immunity in infection is well known, their role in innate defense remains unclear. Pamer and his colleagues (73) showed, for the first time, that dendritic cells can directly exert anti-bacterial immunity without priming T cells. After *Listeria monocytogenes* infection, the CD11b⁺ CD11c^{int} dendritic cell population increased in the spleen, the major site of the infection. Moreover, these cells produce high amounts of TNF α and iNOS; hence, they are called TNF iNOS-producing dendritic cells (TipDC). In CCR2-deficient mice, the dendritic cell population does not increase after infection, which correlates with less TNF α and iNOS production and delayed clearance of the infection even though T cell responses to *L. monocytogenes* were preserved.

Besides infection, monocytes also contribute to sterile inflammation, as in atherosclerosis, which is an inflammatory disease (74, 75) characterized by monocyte infiltration into lipid-laden plaques (76). Just after initiation of high-fat diet, blood leukocytes start attaching to endothelial cells. In a rabbit model of atherosclerosis, endothelial cells express VCAM-1 during early atherogenesis (77). High VCAM-1 expression is confined to activated endothelium lining atherosclerotic plaques. VLA-4 expressed on monocytes can bind with

VCAM-1. Adhesion to endothelial cells may also be mediated by ICAM-1, which binds to LFA-1 expressed by monocytes. Once monocytes attach to arterial endothelial cells, they can enter the intima by diapedesis at the junction of neighboring endothelial cells. This process is mediated by chemoattractant cytokines such as MCP-1, and MCP-1 mRNA has been detected in human and rabbit atherosclerotic plaques (78). In early atherogenesis, the main chemokine sources are vascular smooth muscle cells and endothelial cells, though, lipid-laden foam cells can also make MCP-1. ApoE-deficient mice lacking CCR2, a receptor for MCP-1, exhibit markedly reduced lesion formation and decreased macrophage numbers in the aorta (79), indicating an active role for the MCP-1-CCR2 pathway in monocyte recruitment. In addition to CCR2, Ly-6c^{high} monocytes require CX₃CR1 to accumulate within plaques (56). However, Ly-6c^{low} monocytes depend on CCR5 to enter plaques. Collectively, CCR2-MCP-1, CX₃CR2-CX₃CL1 and CCR5-CCL5 axes are important for monocyte recruitment to plaques (80). Indeed, inhibiting these three pathways in combination almost completely abolished atherosclerosis (81). Interrupting monocyte migration from the blood pool to the atherosclerotic plaque is a promising therapeutic option currently being investigated by multiple groups (82, 83). Our work on RNAi revealed that silencing the chemokine receptor CCR2 in monocytes drastically reduces their migratory capabilities and lowers both monocyte and macrophage numbers in atherosclerotic plaque (52, 84).

Myocardial infarction (MI), another form of sterile inflammation, is the most frequent cause of death in the USA (85). After MI, dead myocardium must be cleared effectively for proper wound healing. MI triggers recruitment of Ly-6c^{high} monocytes followed by Ly-6c^{low} monocytes (14). Ly-6c^{high} monocyte recruitment is CCR2-dependent, whereas Ly-6c^{low} monocytes are recruited via CX₃CR1. Ly-6c^{high} monocytes digest damaged tissue by releasing proteolytic enzymes and inducing phagocytosis. In contrast, Ly-6c^{low} monocytes may promote healing by encouraging myofibroblast accumulation, collagen deposition and angiogenesis in the ischemic tissue.

Fate of macrophages in tissue

In atherosclerosis, lipoprotein is engulfed by monocyte-derived macrophages that subsequently become foam cells. Oxidative stress in the arterial wall modifies low-density lipoprotein (LDL), which is then taken up by macrophages through scavenger receptors such as SR-A1, MARCO (SR-A2), CD36, SR-B1, LOX1, SREC1, and SR-PSOX (80). The scavenger receptors can recognize and process modified LDL. Free cholesterol is then trafficked to endoplasmic reticulum where it undergoes esterification to form cholesterol fatty acid esters.

The number of macrophages in atherosclerotic plaques is determined by monocyte recruitment and local macrophage proliferation. Monocyte recruitment is dominant in the first 4-8 weeks after initiation of high-fat diet (59), but local macrophage proliferation dominates later. In early atherosclerosis, monocyte recruitment comprises 70% of the plaque macrophage pool, with *in situ* proliferation making up the remaining 30%, whereas in established atherosclerosis, plaque macrophage origins are 87% proliferated monocytes versus 13% recruited.

Plaque macrophage pool size is also determined by cell retention or egress cues (86). In addition to plaque macrophage phenotype, the net kinetics of supply, exit, and/or death decide between atherosclerotic progression and regression. When plaque-enriched aortic arches collected from ApoE^{-/-} mice were transplanted into the abdomen of ApoE^{-/-} mice (progressive atherosclerosis), very few plaque macrophages egressed into iliac lymph nodes (87). In contrast, when aortic arches were transplanted into the abdomen of ApoE^{+/+} mice, substantial numbers of macrophages egressed from plaques, resulting in regression of atherosclerosis. In a regressive environment, the departed macrophages had higher CCR7 mRNA levels than in progressive atherosclerotic disease. When mice with regressive atherosclerosis were treated with antibodies against CCR7 ligands CCL19 and CCL21, macrophage egress was abrogated (88), resulting in unaltered lesion size and foam cell content in the transplanted aortae. Expression of CCR7 depends on liver X receptor α and β (LXR α and β). Macrophages lacking the receptors exhibit significantly less emigration from atherosclerotic plaques (89). Besides these macrophage egress cues, retention cues also regulate plaque macrophage migration. Netrin-1, a neuroimmune guidance molecule expressed by human and mouse macrophages, inhibits migration of macrophages directed by CCL2 and CCL19. Netrin-1 deficiency in hematopoietic cells resulted in reduced atherosclerosis (90). Macrophage egress and retention cues, together with monocyte recruitment, determine the net macrophage content in atherosclerotic plaque. Suppressing monocyte recruitment with ApoE treatment successfully reduced atherosclerotic plaque burden and macrophage content without involvement of macrophage migratory cues (91).

Monocyte and macrophage functions

Macrophages remove apoptotic cells and debris in homeostatic conditions. Autoantigen clearance in homeostasis has various functions, such as preventing autoimmunity by inducing self-tolerance. Kronke and his colleagues (92) showed that resident macrophages take up apoptotic cells in a 12/15-lipoxygenase-dependent manner, which acts as sink for apoptotic cells and blocks their uptake by inflammatory monocytes. Inhibiting 12-15-lipoxygenase led inflammatory monocytes to process self-antigens; subsequently, self-antigens presented to cytotoxic T cells, which resulted in lupus-like autoimmune disease.

Resident macrophages can also participate in tissue or organ regeneration. Adult salamanders can regrow complete body structures, a process that involves myeloid cell accumulation in the wound (93). Systemic myeloid ablation blocked limb generation and led to scarring. Macrophages, which are abundant in the adult heart (16, 94), may have similar roles in heart regeneration (95). Myocardial infarction in adult mice and humans leads to damage and death of cardiomyocytes, which triggers inflammation and clearance of dead cells and facilitates infarct healing and scar formation (96). However, in young zebrafish and neonate mice, the heart can regenerate after injury without scarring (93, 95). Ischemic injury in the neonatal mouse heart, in the presence of abundant macrophages, resulted in complete regeneration, with no scar, while depleting macrophages resulted in incomplete healing with scar formation. Macrophages can also participate in adaptive thermogenesis (97). Upon cold exposure, adipose tissue macrophages undergo IL-4 and IL13-dependent alternative activation, which leads activated macrophages to secrete catecholamines. This induces thermogenic gene expression in brown adipose tissue and lipolysis of white adipose tissue.

As discussed above, monocytes and monocyte-derived macrophages are also important in mediating and resolving inflammation. Myocardial infarction causes an inflammatory reaction (96, 98) that is essential to healing and scar formation. However, excessive inflammation may also increase mortality. Patients with myocardial infarction exhibit high leukocyte count in the blood and the infarct, which impedes infarct healing. Post-MI mortality correlates with white blood cells count. Peak blood monocyte count after MI positively correlates with left ventricular end-diastolic volume and negatively correlates with ejection fraction (99). Excessive inflammation in the infarct after MI, which is very common in patients with atherosclerosis, promotes left ventricular dilation and heart failure. Similarly, high fat diet causes monocytosis in ApoE^{-/-} mice. These monocytes are recruited to atherosclerotic plaques and produce various inflammatory cytokines, such as MPO, MMP2 and MMP9. They can digest extracellular matrix and weaken the fibrous caps overlying plaques, rendering the plaques more vulnerable to rupture.

Systemic monocytosis also contributes to metabolic diseases such as obesity (100) and diabetes (101). Adipose tissue inflammation plays a major role in metabolic diseases (102). Groundbreaking obesity research by Chen (103) and Ferrante (104), for the first time, showed that macrophages accumulate in adipose tissue and play a crucial role in obesity-related insulin resistance. Macrophage accumulation in adipose tissue is CCR2 dependent, as CCR2-deficient mice have reduced adipose macrophages and improved insulin sensitivity (105). In lean mice, 10-15% of cells in adipose tissue express F4/80, a macrophage marker, whereas about 50% of the cells in obese mice are F4/80⁺ (106). Moreover, adipose tissue macrophages in lean mice are the M2 phenotype (ARG1⁺ CD206⁺ CD301⁺) (107), but adipose tissue macrophages in obese mice produce NOS2 and TNF α , indicating an M1 phenotype. Alternative activation of adipose tissue macrophages depends on peroxisome proliferator activated receptor- γ (PPAR γ) (108). Myeloid cell-specific PPAR γ deletion made mice more prone to obesity and insulin resistance.

Resolution of inflammation

As soon as infectious agents are neutralized and dead cells are removed, inflammation must be resolved and tissue homeostasis is reestablished to ensure repair and regeneration. Atherosclerosis has defective resolution of inflammation (109), which leads to vulnerable plaques and myocardial infarction. The process of inflammation is considered to be active rather than passive (110), and its resolution is similarly active. Effective resolution of inflammation involves efficient clearance of apoptotic cells by phagocytes, a mechanism known as efferocytosis. Apoptotic cell death may also instigate resolution. At one week after MI, apoptosis of immune cells in the infarct exceeds leukocyte recruitment, which is followed by phagocytosis of tissue debris by macrophages, resulting in resolution of inflammation (17). Efferocytosis promotes resolution of inflammation by engulfing dying cells capable of releasing inflammatory contents and producing anti-inflammatory mediators such as IL-10 and TGF β (111). Following encounter with apoptotic cells, macrophages exhibit increased expression of lipoxygenase. This promotes production of resolvins and maserins, which are important mediators in inflammation resolution (112). Granulocytes are one of the first cell types to infiltrate injured tissues. Once the 'threat' is abolished, granulocytes die to allow resolution of inflammation. In non-complicated inflammation,

granulocytes undergo apoptosis. Death of granulocytes can also be mediated by nitric oxide produced by alternatively activated (M2) macrophages (110). Additionally, lipid mediators, such as cyclooxygenase (COX)-generated eicosanoid and prostaglandin E2, generate pro-inflammatory, anti-inflammatory and pro-resolution mediators that facilitate resolution of inflammation.

Conclusions

Acute and chronic inflammation results in systemic monocytosis. Monocytes are recruited to inflammatory sites where they mediate inflammation and differentiate into tissue macrophages. Blood monocytosis enables eradication of infection and removal of cellular debris after injury and ischemia. Monocyte-derived macrophages may also support resolution of inflammation, if their phenotype permits. However, exaggerated monocytosis during inflammation likely harms tissues by limiting resolution of inflammation and propagating exaggerated immune activation, for instance in sepsis. Despite growing evidence of inflammation's importance in post-MI left ventricular remodeling and heart failure, specific anti-inflammatory therapy has yet to be implemented. Monocytosis can be therapeutically regulated at different stages: monocyte production in the hematopoietic niche, release from the bone marrow and spleen, recruitment to sites of inflammation and/or macrophage polarization. Stem cell factor (31), IL-1 β (17), IL-3, and G-MCSF (58) are candidate targets for reducing monocytosis following MI and in atherosclerosis. MCP-1, CCR2 and angiotensin-II receptor 1 may be blocked to reduce monocyte release from bone marrow and splenic niches. siRNA or small molecule inhibitors that target adhesion molecules such as VCAM-1, ICAMs, E-selectins, and chemokines such as MCP-1, CX₃CL1, and CCL5 can reduce monocyte recruitment to inflammatory sites. Since alternatively activated macrophages support resolution of inflammation, therapeutics facilitating M1-to-M2 macrophage phenotype transition, for example by inhibiting the transcription factor IRF5, will resolve inflammation more quickly (55). Tissue healing and resolution of inflammation require balanced pro- and anti-inflammatory responses. Monocytes and macrophages secrete pro-inflammatory cytokines that can render atherosclerotic plaques vulnerable to rupture, promote heart failure and support insulin resistance in type II diabetic patients. On the other hand, alternatively activated macrophages stimulate tissue repair by encouraging collagen production, bolster angiogenesis and secrete anti-inflammatory cytokines that can increase insulin sensitivity in lean animals. This emerging complexity of the innate immune system implies that we must develop therapies that precisely target the right cells at the right time to maximize benefits and limit collateral damage.

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Fig. 1. Monocytoysis after acute and chronic inflammation

Infection and sterile inflammation, such as myocardial infarction, decrease production of hematopoietic stem cell (HSC) retention factors by hematopoietic niche cells. This leads to increased HSC cycling resulting in elevated hematopoiesis. HSC-derived monocytes in the bone marrow can intravasate into blood circulation in an MCP-1-dependent manner. HSCs can also circulate in the blood and lodge in the spleen, where they produce monocytes in presence of SCF, G-MCSF, IL-3, and IL1 β . Release of splenic HSC into the blood depends on AT-II-AT1r signaling. Finally, blood monocytes are recruited to sites of inflammation, where they differentiate into macrophages with several functions, including inflammation, healing, and resolution of inflammation.

Table 1

Protein/transcription factors that regulate HSC commitment towards different hematopoietic lineages

Protein/ Transcription factor	Gene symbol	Function	Reference
CCAAT/enhancer-binding protein alpha	Cebpa	Committed B and T lymphoid cells can be reprogrammed to functional macrophages through C/EBPalpha expression.	6, 42, 43
PU.1	Sfpi1	PreT cells can be reprogrammed to myeloid dendritic cells through PU.1 expression	6
Erythroid transcription factor	Gata-1	Gata-1 complements PU.1 and inhibits myeloid differentiation (i.e. promotes erythroid/ megakaryocytic/ eosinophil differentiation).	6, 48
Early growth response gene-1	Egr-1	Egr-1 induces by PU.1 and can selectively induce macrophage differentiation.	44, 45, 46
IFN consensus sequence binding protein	Irf8 (Icsbp)	Drives monocyte differentiation.	44, 47
Kruppel-like factor	Klf4	Selectively rescues monocyte differentiation of PU.1 ^{-/-} progenitors.	113
V-maf musculoaponeurotic fibrosarcoma oncogene homolog B	Mafb	Inhibits erythroid differentiation and induces monocyte commitment of human hematopoietic stem and progenitor cells.	115
V-maf musculoaponeurotic fibrosarcoma oncogene homolog	Maf (Maf2)	Induces monocyte differentiation from bipotent myeloid progenitors.	114