Macrophages are the building blocks of intimal atherosclerotic lesions where they scavenge and accumulate modified lipoproteins, profoundly affecting lesion development and progression through their roles in lipid metabolism and as major sensors and effectors of the innate immune system. Lesional macrophages originate from circulating monocytes, although more recent work indicates that a subpopulation may also derive from dedifferentiated vascular smooth muscle cells. Resident macrophages are found in the heart at steady state and along with newly recruited monocyte-derived macrophages accumulate after myocardial infarction. Macrophage turnover in atherosclerotic lesions and ischemic myocardium is under the control of several biological processes, including monocyte recruitment, macrophage proliferation, and apoptosis, as well as macrophage egress. Moreover, local microenvironment whether in health or disease profoundly affects macrophage phenotype and consequently, both metabolic and immune-related responses. Here, I summarize recent work published in ATVB on the roles of monocytes/macrophages in cardiovascular diseases (CVDs) and review it in light of the most recent advances in this research area.

Monocytes, Macrophages, and the Pathogenesis of Atherosclerosis

Monocyte Activation/Recruitment and Macrophage Trapping Within the Lesions

Monocytes play central roles in atherogenesis and are rapidly attracted to sites of disturbed flow characterized by low-grade inflammation. The use of advanced 11-fluorochrome, 13-parameter flow cytometry method recently allowed detailed characterization of the dynamics of immune cell accumulation during flow-induced (partial carotid artery ligation) atherogenesis in apolipoprotein E-deficient (ApoE−/−) mice. The study showed that monocytes/macrophages constitute the most abundant cell population in the intima where they rapidly accumulate as early as 4 days after induction of flow disturbance, peak at day 7, and remain relatively stable between days 14 and 28. Disturbed flow promotes endothelial cell activation in part through repression of major antiadhesive and antithrombotic transcription factors, Klf2 and Klf4. In fact, a single microRNA, mir-92a, coregulates Klf2 and Klf4 expression and contributes to the heterogeneity of endothelial cell phenotype in atheroprone versus atheroresistant arterial sites. Hypercholesterolemia combined with low shear stress markedly upregulates endothelial expression of mir-92a in a Stat3-dependent manner. Blockade of mir-92a restores Klf2 and Klf4 expression, reduces endothelial inflammation and monocyte infiltration, thereby limiting atherosclerosis. Disturbed flow and other proatherogenic factors also downregulate the endothelial expression of negative guidance cues, particularly Netrin-1 and Semaphorin3A, while upregulating EphrinB2, and facilitate chemokine-directed migration of monocytes and their infiltration within the intima. Interestingly, Klf2, Klf4, and Netrin-1 are also expressed in monocytes/macrophages and regulate their phenotype/functional during atherogenesis. Klf2 and Klf4 limit monocyte/macroage activation in part through the regulation of selective microRNAs (mir-124a, mir-150) and promote an alternatively activated M2 phenotype associated with reduced susceptibility to atherosclerosis. However, Netrin-1 expression in macrophages, which is highly upregulated by hypoxia-inducible transcription factor-1α, promotes the progression of atherosclerosis through its profound impact on macrophage migration, limiting their emigration and retaining them within the developing atherosclerotic lesions. The same group recently extended this concept to another guidance molecule, Semaphorin3E, and showed that it is upregulated in M1 macrophages where it blocks actin polymerization and migration in response to chemokine stimulation, thereby contributing to macroage retention within the lesions. Of note, low expression of Netrin-1 and Unc5b in macrophages and smooth muscle cells of human atherosclerotic lesions was associated with signs of plaque instability.

Recent studies have re-emphasized the subtle but crucial roles of post-translational glycosylation of endothelial cell or leukocyte ligands and receptors involved in cell–cell interactions. Disturbed flow and proinflammatory mediators increase hypoglycosylated, high-mannose and hybrid N-glycans on the endothelial cell surface at sites of early lesion development, which leads to increased monocyte adhesion. However, sialyltransferase IV activity, which catalyzes the formation of a sialylated sLeX, seems to promote monocyte recruitment into inflamed arteries and promotes atherogenesis through its essential role in the generation of a functional Ccr5 (C-C chemokine receptor) receptor, suggesting that selective targeting of these post-translational events may constitute an interesting therapeutic option. In addition to Ccr5, signaling events through Cxcr2 and Cx3cr1 (CX3C chemokine receptor) play nonredundant roles in atherosclerosis. Interestingly, more recent studies on Cx3cr1 suggest upregulation of this chemokine receptor on platelets in response to hyperlipidemia.
which promotes platelet–monocyte complex formation and supports monocyte arrest on inflamed vascular cells, thereby identifying a new mechanism leading to platelet accumulation and monocyte recruitment at sites of arterial injury.\(^4^5\) Pharmacological targeting of Cx3c1r through the administration of an amino terminus–modified Cx3c1r ligand endowed with antagonist activity substantially limits monocyte trafficking into lesions and halts the development and progression of atherosclerosis.\(^4^4\)

Monocyte differentiation into macrophages is a pivotal process in atherogenesis and is regulated through a coordinated developmental program dependent on the balance between several transcription factors, including MafB, c-Maf, Iscbp/Irf8, Klh4, Pu.1, and the macrophage-spicifying zinc finger transcription factors Egr-1 and Egr-2.\(^3^6,3^7\) A recent study published in *ATVB* proposed caveolin-1, the structure protein of caveolae, as a critical regulator of monocyte differentiation through extracellular signal-regulated kinases (Erk) phosphorylation, leading to increased Egr-1 nuclear translocation and transcriptional activity.\(^3^8\) A broader view of the molecular mechanisms controlling monocyte activation and priming toward the macrophage phenotype is emerging. Recent studies identified distinct dynamic epigenetic, transcriptional, and metabolic programs controlling monocyte differentiation toward either tolerant or activated trained phenotypes.\(^3^9,4^0\) This is consistently with a highly focused work showing that brief exposure of monocytes to oxidized low-density lipoprotein (LDL) increases trimethylation of lysine 4 at histone 3 in promoter regions of several proinflammatory mediators and scavenger receptors (CD36, SR-A) leading to foam cell formation and long-term proinflammatory cytokine production\(^4^1\) via epigenetic reprogramming. Such sustained changes in monocyte/macrophage phenotype may further promote and sustain immune cell activation. For example, CD36-dependent activation of monocytes generates an intracellular transduction pathway leading to Rac and Jnk2 activation downstream of the guanine nucleotide exchange factor Vav1, which contributes to enhanced monocyte adhesion to inflamed endothelium.\(^4^2\) This is reminiscent of the role of CD36 in the trapping of macrophages within atherosclerotic lesions previously shown to be dependent on alteration of cytoskeletal dynamics through sustained activation of Fak and reduced migration.\(^4^3\) Interestingly, AT1 receptor activation may upregulate CD36 expression on macrophages, sustain Fak activity, and contribute to macrophage trapping in the intima, leading to heightened plaque inflammation and increased susceptibility to rupture.\(^4^4\) In this regard, a more recent study indicates that angiotensin-converting enzyme, the enzyme that converts angiotensin I (AngI) into AngII, is highly expressed in lesional macrophages where it contributes to lesion development,\(^4^5\) although the effect was modest and limited to the aortic arch. Superimposed metabolic stress factors, like high LDL and d-glucose concentrations may further prime monocytes and enhance their chemotaxis through increased S-glutathionylation and remodeling of actin downstream of NADPH oxidase 4. In contrast, regulators of lipid metabolism such as Apoe may suppress atherosclerosis through reduced lipid accumulation in monocytes and downregulation of monocyte activation, independently of their lipid-lowering properties.\(^4^6\)

More on Foam Cell Formation and Macrophage Activation/Deactivation

Monocyte differentiation into macrophages is associated with upregulation of phagocytic activity leading to lipid accumulation and formation of typical foam cells. Recent findings suggest that cardiovascular risk factors other than elevated plasma cholesterol levels may significantly modulate macrophage foam cell formation. For example, the endogenous nucleoside adenosine, which is released extracellularly under stress conditions, profoundly affects foam cell formation through G-protein–coupled receptor A(2A)–dependent regulation of reverse cholesterol transport.\(^4^7\) Increased expression of xanthine oxidoreductase, a key enzyme in the uric acid production pathway, is upregulated in many CVD settings and localizes to macrophages. Recent data indicate that overexpression of xanthine oxidoreductase promotes foam cell formation through upregulation of very LDL and scavenger receptors and downregulation of ATP-binding cassette transporters (ABCA1 and ABCG1) along with induction of a proinflammatory phenotype, all effects being prevented by treatment with allopurinol, which markedly limits lipid accumulation and lesion calcification in aortas of Apoe\(^-/-\) mice.\(^4^8\) Hypertriglyceridemia is an important cardiovascular risk factor and triglyceride-mediated pathways are causally related to coronary disease.\(^4^9\) Bojic et al\(^5^0\) show that very LDL treatment of macrophages leads to triglyceride accumulation and induces AP1-dependent proinflammatory cytokine production, downstream of Erk1/2 and Akt/Foxo1. PPARδ agonists attenuated very LDL–stimulated triglyceride accumulation through reduction of lipoprotein lipase activity, increased fatty acid uptake and enhanced β-oxidation, and inhibited very LDL–dependent inflammation through normalization of Erk and Akt/Foxo1 signaling, which led to an M2 macrophage phenotype and limited the progression of pre-established atherosclerosis in mice.\(^5^1\) Angiopoietin-like 4 (ANGPTL4) is an endogenous inhibitor of lipoprotein lipase. Nonsynonymous variants in ANGPTL4 were initially associated with reduced triglyceride levels and increased high-density lipoprotein.\(^5^2\) However, subsequent larger studies failed to establish a correlation between APNTL4 variants or plasma levels and serum triglycerides, although plasma ANGPTL4 negatively associated with high-density lipoprotein cholesterol.\(^5^3\) Therefore, an uncertainty remained on the potential impact of such variants on CVD. A recent work in *ATVB* clearly shows that transgenic overexpression of Angptl4 suppresses foam cell formation and significantly reduces atherosclerosis in mice despite no alteration of plasma cholesterol or triglycerides levels.\(^5^4\) Supporting a protective effect of Angptl4 and a potential impact of ANGPTL4 variants on CVD beyond any role on serum lipid levels.

Macrophage foam cell formation and inflammation are prominent features of atherosclerotic lesions. However, recent work indicates that cholesterol accumulation does not invariably lead to inflammation. In fact, analysis of elicited peritoneal macrophages in wild-type and *Ldlr\(^-/-\)* mice fed...
either a chow or a high-fat high-cholesterol diet revealed massive reprogramming of the lipidome in response to both diet and genotype and an unexpected deactivation of the inflammatory response in the macrophage foam cells.55 The underlying mechanisms involved regulated accumulation of desmosterol in foamy macrophages, which was responsible for downstream activation of LXR (liver X receptor) and inhibition of SREBP (sterol regulatory element-binding protein) target genes, leading to selective reprogramming of fatty acid metabolism and the establishment of an anti-inflammatory homeostatic response.55 In line with these observations, Suzuki et al56 were unable to detect any evidence for increased expression of inflammatory proteins after in vitro loading of peritoneal macrophages with acetyl-LDL and demonstrated that sterol loading blunted the macrophage response to lipopolysaccharide (LPS) without altering the overall pattern of LPS-induced gene expression. However, their use of transcriptomics and proteomics to investigate molecular changes induced in cholesterol-loaded macrophages revealed a differential activation, mainly through post-transcriptional mechanisms, of 3 functional modules corresponding to lipid metabolism, lysosomal biology and, unexpectedly, complement activation, both the classic and the alternative pathways.56 Thus, despite no direct induction of an inflammatory program in foam cell macrophages, complement regulation by sterol loading may have important consequences on the immune response and the development of atherosclerosis.57 It should also be noted that enrichment of monocyte/macrophages with unesterified cholesterol generates biologically active phosphatidylserine-expressing microvesicles that may carry not only a thrombotic potential but also danger signals such as peroxides, induced through activation of mitochondrial permeability transition pore 

Innate Inflammatory Signaling Pathways

An intriguing role for TL7/9 (toll-like receptor) signaling in foam cell formation has been reported recently59 beyond its role in macrophage activation. However, the molecular mechanisms behind this observation remain to be elucidated. Other work has expanded on the role of inflammatory signaling in macrophages as a major driver of atherogenesis. Some studies proposed a role for NOD2 (nucleotide-binding oligomerization domain-containing protein)-mediated activation of macrophages within the lesions, leading to p38-dependent activation of COX-2 (cyclooxygenase) through upregulation of proinflammatory cytokines interleukin (IL)-1β and tumor necrosis factor-α and culminating in increased eicosanoid production (mostly prostaglandin E2).60 Macrophages can also be primed for increased eicosanoid (including PGE2 [prostaglandin E]) secretion after LXR-mediated induction of lysophosphatidylcholine acyltransferase 3.61 In this regard, suppression of myeloid derived, but not vascular cell derived, PGE2 substantially reduces atherosclerosis.62 Other studies confirmed a pivotal role for nuclear factor (NF)-κB pathway, and more particularly myeloid-specific IκB kinase activity, in sustaining macrophage inflammation and promoting lesion development.63 A number of interesting inflammatory pathways that converge on NF-κB activation have been reported. Sphingomyelin generation in macrophages through sphingomyelin synthase 1 promotes atherosclerosis through accentuation of TLR4-dependent NF-κB signaling.64 TWEAK (tumor necrosis factor-like weak inducer of apoptosis)-mediated activation of monocytes/macrophages through fibroblast growth factor inducible 14 increases the release of the proinflammatory and proatherogenic HMGB1 (high-mobility group protein B),65,66 and upregulation of resistin-like molecule-β on foam cell macrophages by saturated fatty acids potentiates the classical NF-κB pathway and accelerates lesion development.67 An additional intriguing mechanism of macrophage activation involves interference with extracellular matrix remodeling through activation of heparanase activity, which cleaves heparan sulfate side chains. Blich et al68 nicely showed that heparanase potently activated macrophages to upregulate tumor necrosis factor-α, IL-1, CCL-2 and MMP-9 (matrix metalloproteinase), likely through TLR2/4, PI3K (phosphoinositide 3 kinase), MAPK (mitogen-activated protein kinase), and NF-κB signaling pathway. How heparanase activates TLRs is still unknown but may involve conformational changes in cell membrane heparan sulfate proteoglycans, generation of heparan sulfate-cleavage products, or other heparan sulfate-independent function. Of note, vulnerable coronary plaques showed increased heparanase activity compared with stable lesions, and elevated plasma heparanase levels were found in patients with acute myocardial infarction compared with stable patients, highly suggesting a clinically relevant pathway.68 Metabolic disturbances associated with obesity and insulin resistance drive cardiovascular complications.69,70 Free fatty acids are elevated in patients with the metabolic syndrome and are proposed to promote inflammatory responses through TLR and NF-κB–dependent signaling. Recent studies provided additional mechanisms for free fatty acid–dependent regulation of the inflammatory response. Saturated free fatty acid palmitate induces the activation of NLRP3 (NOD-like receptor family, pyrin domain containing 3)-ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD) inflammasome leading to increased production of IL-1β (and IL-18),71 whereas unsaturated oleate selectively induces inflammasome-independent IL-1α production through activation of mitochondrial uncoupling.72 Recent work published in ATVB indicates that stearic acid, a major saturated free fatty acids in atherosclerotic lesions, activates TLR2/4-independent inflammation in macrophages, with induction endoplasmic reticulum stress and macrophage apoptosis,73 suggesting potentially important consequences with regard to lesion progression, given the well-documented roles of endoplasmic reticulum stress, macrophage apoptosis, and defective efferocytosis in promoting plaque inflammation and necrotic core formation.74-76 Conversely, n-3 fatty acids are endowed with anti-inflammatory properties. For example, eicosapentaenoic acid has been shown recently to limit the induction of macrophage endothelial lipase in response to a variety of stimuli, including LPS, palmitic acid, or PPARγ agonists. The effect was associated with reduced production
of proinflammatory mediators but upregulation of antiinflammatory IL-10.77 Moreover, administration of ω-3 fatty acids to Ldlr−/− or Apoe−/− mice led to significant reduction of Ly6C+ monocytes and monocyte recruitment into developing lesions, independently from effects on plasma cholesterol.78 More generally, ω-3 fatty acids were recently shown to uncouple NF-κB binding and histone acetylation at enhancers and promoters from subsequent steps required for induction of inflammatory genes, particularly histone methylation and eRNA (enhancer RNA) production.79

Intraplaque hemorrhage is an important risk factor for plaque progression and vulnerability where erythropagocytosis by macrophages seems to play a determinant role.80,81 However, the mechanisms behind this increased susceptibility to atherosclerosis are poorly understood. Two studies published in ATVB suggest a critical role for hepcidin, a key regulator of iron homeostasis, in driving foam cell formation (in part through modulation of ABCA1 and ABCG1 expression), oxidative stress, and production of proinflammatory cytokines by macrophages, particularly in association with erythropagocytosis.82,83 highlighting a mechanistic link between iron retention in macrophages, inflammatory activation, and lesion progression.

Anti-Inflammatory Signaling Pathways

Intraplaque hemorrhage has also been shown to generate a distinct adaptive macrophage state in lesions84 dependent on a key transcription factor ATF1 induced by heme.85 and the associated induction of LRX and HO-1 target genes.86,87 Heme-induced HO-1 in macrophages requires NRF-2 (nuclear factor [erythroid-derived 2]-like),88 recently shown to prevent oxidative stress in these cells, leading to limitation of plaque inflammation and progression.89 Moreover, activation of 5′-AMP–activated protein kinase is instrumental in heme-induced ATF1 and downstream suppression of macrophage oxidative stress and protection from foam cell formation.90 This may provide potential explanation for the vasculoprotective effects of the 5′-AMP–activated protein kinase activator metformin and its unique ability to reduce the macrovascular complications of diabetes mellitus91 among many other anti-diabetic treatments.Remarkably, 5′-AMP–activated protein kinase activation by metformin was recently shown to suppress trained immunity in macrophages through inhibition of mTOR (mammalian target of rapamycin),92 revealing a potentially broader role for this pathway in the regulation of macrophage immune activation versus tolerance.

Macrophage polarization is proposed to play determinant roles in CVDs and the topic has been the subject of many recent reviews in ATVB.91–99 Additional original work further highlighted the mechanisms and the impact of macrophage polarization on atherosclerosis. For example, deficiency of Klf4 in macrophages limits M2 and promotes a proatherogenic M1 phenotype,90 whereas control of oxidative stress by thioredoxin is reported to favor the development of an atheroprotective M2 phenotype.91 M2 polarization is dependent on fatty acid oxidation. Interestingly, cell-intrinsic lysosomal lipolysis by lysosomal acid lipase seems to be essential for the induction of a coordinated program leading to alternative activation of macrophages.100 Autophagy has been shown previously to regulate cholesterol efflux in macrophages through targeting of lysosomal acid lipase to lipid droplets with subsequent hydrolysis of cholesteryl esters and generation of free cholesterol for ABCA1-dependent efflux.101–103 However, no evidence is available yet to directly implicate autophagy in lysosomal acid lipase–dependent induction of an M2 phenotype, although it plays pivotal roles in macrophage homeostasis, limiting oxidative stress, inflammasome activation, and promoting effector cytokis and antiatherogenic pathways.104,105 Additional studies expanded on the role of TIMP3 (tissue inhibitor of metalloproteinases) in macrophage biology based on previous work that highlighted the invasive potential of TIMP3(−)MMP14(+) foam cell macrophages.106 The new results show that TIMP3 is upregulated by classic but downregulated by alternative macrophage activation107 and that overexpression of TIMP3 in macrophages leads to reduction of oxidative stress, decreased inflammation, and a stable plaque phenotype.108 The exact mechanisms behind this observation remain to be elucidated. Additional work on the consequences of PPARγ activation in macrophages revealed selective activation of 11β-hydroxysteroid dehydrogenase type 1 in M2 versus M1 macrophages.109 Uprregulation of 11β-hydroxysteroid dehydrogenase type 1 would increase conversion of cortisone into active cortisol. The authors suggested that this might promote improved resolution of the inflammatory response. However, direct inhibition of 11β-hydroxysteroid dehydrogenase type 1 or its deletion in bone marrow–derived cells does not promote but rather limit atherogenesis through reduction of inflammatory macrophage phenotype and improved cholesterol ester export.110 Thus, additional work is needed to better clarify the relevance of PPAR-γ-induced 11β-hydroxysteroid dehydrogenase type 1 in M2 macrophages. Finally, a new anti-inflammatory mechanism for apoAI mimetic peptide 4P was reported, involving disruption of lipid rafts, reduced recycling of TLR4 after LPS stimulation, and inhibition of NF-κB–dependent proinflammatory gene expression.111

Macrophages in Aortic Aneurysm

Abdominal aortic aneurysm (AAA) is characterized by extensive remodeling of the arterial wall, leading to wall thinning, weakening, dilatation, and rupture.112 Despite extensive studies implicating the inflammatory response in AAA,113 little is known about the role of monocyte/macrophages in disease pathogenesis. Monocyte depletion reduces AAA formation in mice.114 However, the distinct contributions of the various monocyte/macrophage subsets to AAA are still relatively unexplored. A few recent studies published in ATVB started to address some of these issues. Two studies highlighted the important role of chemokine-dependent monocyte recruitment through Ccr2 or Cxcl4 (CXC chemokine ligand)–Cxcl5 in the development of AAA115,116 using AngII-dependent or elastase-induced models.117 The studies suggested new therapeutic medical strategies for this disease using peptide-mediated inhibition of Cxcl4–Cxcl5 interactions115 or treatment with everolimus to inhibit AngII-induced interferon-γ production and interferon-γ–induced Ccr2 expression on monocytes.116 A note of caution however should be added
for everolimus, recently shown to trigger cytokine release by macrophages through mTOR inhibition and activation of p38 Mapk. Additional studies addressed the impact of modulation of macrophage functions on the development of AAA. Haploinsufficiency of Notch1 in bone marrow–derived cells reduced the incidence of AngII-induced AAA in Apoe−/− mice, which was associated with reduced aortic influx and accumulation of macrophages, reduced macrophage migration and proliferation in vitro, and a switch toward an M2 phenotype. The results extend previous work that implicated Notch1 signaling in the pathogenesis of aortic valve calcification/bicuspidy and thoracic aortic aneurysm. Another study addressed the role of macrophage-derived Angptl2 in the pathogenesis of AAA. Angptl2 expression is known to be induced in several inflammatory settings, including obesity-related insulin resistance and is particularly associated with smoking, the dominant risk factor for AAA. Deletion of Angptl2 in bone marrow–derived cells led to protection from CaCl2-induced AAA, which was associated with reduced macrophage activation and blunted MMP9 production, suggesting a major impact on vascular inflammation, probably through inhibition of IkB degradation and matrix remodeling. The relevance of macrophage phenotype to AAA pathogenesis in humans is exemplified in 1 study that used advanced laser capture microdissection to characterize macrophage subsets in aortic tissue and showed that different macrophage subsets localized to distinct regions in the aneurysmal tissue, with preferential localization of an inflammatory CD68(+)mannose receptor(−) subset to adventitia, whereas the intraluminal thrombus predominantly harbored an anti-inflammatory CD68(+)(MR(+)) macrophages. Interestingly, the inflammatory subset was enriched in peroxiredoxin-1, suggesting a predominant contribution to oxidative stress in AAA and a potential role in AAA development and progression, given that serum peroxiredoxin-1 levels positively correlate with AAA size and growth rate.

### Macrophages in Heart Diseases

The past few years witnessed important progress in our understanding of the immune response to ischemic injury, and more particularly the role played by the various subsets of monocytes/macrophages in this process. Selective macrophage subsets (mainly of yolk-sac origin) populate the heart at steady state, are capable of self-renewal, and probably play a role in immune surveillance. Atrial injury, the contribution of monocytes and monocyte-derived macrophages become predominant with distinct pathogenic and protective roles for Ly6Cmi and Ly6Chi monocyte subsets, respectively, but still poorly defined roles for the newly accumulated macrophage subsets in coordinating inflammation versus reparative processes. A few recent studies published in ATVB examined the regulation and function of macrophages in response to heart injury. Deletion of the chemokine decoy receptor D6, which binds and selectively inactivates inflammatory CC-chemokines, led to larger infarcts and increased incidence of cardiac rupture in mice subjected to coronary artery ligation. The results were attributed to heightened Ccl2/3 activity, increased accumulation of neutrophils and Ly6Chi monocytes in the ischemic heart, along with upregulation of inflammatory signaling and Mmp2/9 activities. The whole phenotype was reversed after reconstitution of D6−/− animals with Ccr2-deficient bone marrow, clearly implicating leukocyte-selective Ccr2 signaling in the myocardial response to ischemic injury. Deletion of Irak-M, a functional decoy that lacks endogenous kinase activity and inhibit Tlr and I1-1 responses, resulted in enhanced postischemic inflammation and adverse myocardial remodeling. The effects were attributed to direct regulation of MMPs and inflammatory signaling in cardiac fibroblasts, which led to secondary accumulation of Ly6Cm monocytes. Two studies addressed the role of macrophage phenotype in mediating the cardiac fibrogenic effects of AngII. Both studies concluded to an important role of M2 induction in this process, which stimulated cardiac fibroblasts to differentiate into α-smooth muscle actin–positive and collagen I–positive myofibroblasts. One study identified a requirement for serum glucocorticoid kinase 1 in promoting optimal Stat3 phosphorylation downstream of AngII, favoring the differentiation toward M2. In the other study, the authors unraveled an interaction between CD4+ T cells and macrophages that coordinated the induction of the M2 phenotype. Deletion of Il-12p35 in macrophages favored T-cell differentiation toward Th2, which in turn was required for an optimal switch toward the M2 phenotype.

### Macrophages as Diagnostic, Prognostic, or Therapeutic Targets in CVD

#### Macrophages as Imaging Targets

Noninvasive in vivo visualization of inflammatory processes within vascular lesions or injured tissues may allow better classification of patients at high risk of cardiovascular events. Nonconjugated ultrasmall superparamagnetic iron oxide (USPIO) particles allow the detection of phagocytic cell activity and have shown promise in identification of inflammatory vascular lesions. In a recent study, Hasan et al have extended the methodology to the detection of inflammatory cerebral aneurysms in humans using ferumoxytol-enhanced MRI and showed selective uptake by CD68(+) macrophages. Three other groups developed MRI protocols and used functionalized USPIO or micron-sized paramagnetic iron oxide (MPIO) particles to target cells expressing vascular cell adhesion molecule-1 within atherosclerotic lesions of Apoe−/− mice. Significant plaque enhancement was detected with the functionalized compared with the uncoupled particles. Moreover, histological analyses revealed that iron localized not only to endothelial cells but accumulated mostly in cap and necrotic core macrophages when using USPIO to target vascular cell adhesion molecule-1, suggesting a high potential for this technique to identify vulnerable lesions. The Choudhury group in fact produced dual-ligand MPIO targeted to both vascular cell adhesion molecule-1 and P-selectin and imaged mice using a 9.4-T magnet. The investigators showed selective targeting of atherosclerotic lesions using the functionalized MPIO and histological analysis revealed selective localization of MPIO to endothelium overlying atherosclerotic lesions, with no MPIO detected within lesions, indicating no or minimal uptake by macrophages. Nevertheless, dual labeling with vascular cell...
adhesion molecule-1 and P-selectin directly correlated with the extent of macrophage accumulation within the lesions during a time course of 30 weeks (with a peak of macrophage accumulation at 20 weeks of age), suggesting that imaging of activated endothelium can be used as a good surrogate of lesional macrophage content. The results nicely support the concept of macrophage accumulation being highly dependent on continuous monocyte recruitment but do not really fit with the idea that macrophage proliferation within the lesions plays a pivotal role in lesion progression. To directly image macrophages within lesions, Biessen’s group designed and validated a strategy to detect cells expressing SR-AI, a scavenger receptor abundantly expressed by macrophages. Injection of USPIO conjugated with a specific peptidic SR-AI ligand into humanized Ldlr−/− mice led to a 3-fold improvement in contrast-to-noise ratio of atherosclerotic lesions in comparison with mice injected with nontargeted USPIO or mice with leukocyte SR-AI deficiency, suggesting an interesting strategy to target phagocytic activity in atherosclerotic lesions. In another work by Burtea et al., the authors used USPIO particles conjugated with a linear hexapeptide R826 to target phosphatidylserine, a biomarker of apoptosis. Remarkably, injection into Apoe−/− produced specific negative enhancement of plaques rich in macrophages and neutral fat after visualization using a 4.7-T Burker MRI, again suggesting a potentially useful tool to track vulnerable plaques.

Additional imaging modalities are being tested, including intravascular optical coherence tomography using near-infrared light, which provides images within 10- to 20-μm axial resolution and recently shown to discriminate macrophage rich areas within Apoe−/− lesions. Others have used multispectral and multimodal intravital fluorescence molecular imaging to detect and quantify the inflammatory process elicited in response to (FeCl3-induced) deep vein thrombosis in mice and assess the impact of deep vein thrombosis on subsequent deep vein thrombosis resolution. Their work demonstrates the feasibility of integrated multitarget imaging modality using macrophage and MMP activity fluorescence imaging agents in this setting to detect and quantify the intensity of thrombus inflammation and predicts the magnitude of subsequent deep vein thrombosis resolution.

Monocytes/Macrophages as Prognostic Targets

There is growing interest in the study of the relationship between murine and human monocyte/macrophage subsets, and more generally whether knowledge accumulated from animal studies can be extrapolated to the human setting. About monocytes and CVD, it is interesting to note that the few human translational studies conducted to date have provided support to the experimental data. Circulating levels of classical CD14++CD16− and intermediate CD14++CD16+ monocytes, but not CD14−CD16++ nonclassical monocytes, have been independently associated with cardiovascular events (death, myocardial infarction, and stroke) at follow-up in 2 relatively large cohorts of coronary patients or individuals with no prevalent CVD. In further support of the use of monocytes/macrophages as biomarkers of CVD in humans, Reiner et al. convincingly reported an independent association between circulating levels of soluble CD14 and incident cardiovascular events or all-cause mortality in a cohort of >5000 European-American and black older adults and identified new genetic determinants of sCD14. However, there was no evidence of association between CD14 genotype and CVD risk, suggesting no causal relationship.

Monocytes/Macrophages as Therapeutic Targets

Preliminary data suggest that inhibition of PAI-1/LRP1 interactions using a small molecule inhibitor impairs macrophage migration and limits macrophage accumulation in a model of renal injury. The relevance of the finding to atherosclerosis is still uncertain as macrophage LRp1 regulates efferocytosis and plays a major homeostatic role in vessels, preserving vascular integrity. Two initially lipid-targeting strategies have now been shown to limit monocyte/macrophage-related inflammatory processes, independently from their effects on lipid metabolism. Treatment with ω-3 fatty acids is shown to limit atherosclerosis through reduction of Ly6C+ monocyte recruitment and nicotinic acid may activate GPR109A to limit NF-κB-dependent monocyte activation, chemotaxis, and adhesion on inflamed endothelium in vitro. This is of high relevance to atherosclerosis, as GPR109A is expressed in lesional macrophages (although it may be downregulated in foam cells) and nicotinic acid has been shown recently to limit lesion progression in a GPR109A-dependent manner using Ldlr−/− mice. In further support of the anti-inflammatory properties of nicotinic acid/GPR109A axis, GPR109A signaling promotes anti-inflammatory properties in colonic macrophages and dendritic cells (high production of IL-10) in vivo and favors the differentiation of Tr1-like IL-10-producing T cells endowed with homeostatic properties. Thus, targeting GPR109A signaling in monocytes, macrophages, and dendritic cells may be useful in limiting maladaptive innate and acquired immune responses in CVD.

Macrophages in AAA can readily be imaged with the modified glucose 18 fluoro-deoxyglucose by positron emission tomography imaging, which is associated with enhanced expression of glucose transporters 1 and 3. Interestingly, administration of glycolysis inhibitor 2-deoxyglucose significantly attenuated AAA formation in 2 mouse models of the disease, which was associated with reduced monocyte/macrophage inflammation, MMP production and survival/proliferation. This agrees in general with the role of glycolysis in supporting trained immunity in macrophages and dendritic cells and could be extended to adaptive effector immune cells. Thus, glycolysis inhibition might prove to be a viable therapeutic option to limit pathogenic immune responses in CVD.

Diagnostic and imaging modalities are being combined with therapeutic agents leading to the development of a new research area using theranostics. A few examples have been published recently in ATVB. Maiseyeu et al. showed that gadolinium nanoparticles conjugated with a neutralizing antibody against myeloid-related protein (Mrp)-8/14 (a secreted protein previously shown to promote leukocyte recruitment) limit Mrp-8/14-dependent inflammatory activation of bone marrow–derived macrophages in vitro and selectively image
inflammatory cells in lesions of Apoe\textsuperscript{−/−} mice, suggesting a potential theranostic approach for atherosclerosis. Others have developed photodynamic therapy to image and kill activated and detrimental macrophages within atherosclerotic lesions,\textsuperscript{157} using a combination of photosensitizers and light illumination. In particular, the authors developed a cathepsin B-activatable theranostic agent, which becomes fluorescent and generates singlet oxygen on protease conversion followed by light therapy. They showed selective imaging of macrophage-dependent cathepsin B activity in lesions of Apoe\textsuperscript{−/−} mice, followed by significant reduction of macrophage content after application of light therapy, mostly because of macrophage apoptosis.\textsuperscript{157} Future work should define the use and potential undesirable effects of this attractive see-and-treat approach. Induction of massive macrophage apoptosis in advanced lesions might promote necrotic core formation in the absence of effective efferocytosis pathways.

Conclusions

Inflammatory processes are central to the pathogenesis and complications of CVD. Yet, no anti-inflammatory strategy has been approved for the treatment of patients with CVD, and a few have even failed,\textsuperscript{158,159} although the inefficacy of non-selective sPLA2 inhibitors could have been predicted from both animal\textsuperscript{80} and human Mendelian randomization studies.\textsuperscript{160} This highlights the urgent need for a better understanding of the cellular and molecular determinants of the inflammatory processes related to CVD and the assessment of their clinical relevance. The past few years have witnessed an immense advance in the characterization of the origins and distinct functions of monocyte/macrophage subsets as well as their differential roles in health and disease, and investigators have proposed ingenious strategies to track these cells and influence their behavior in vivo. The day is getting closer when their behavior in vivo. The day is getting closer when

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