Monocyte-Macrophages and T Cells in Atherosclerosis

Ira Tabas[1,*] and **Andrew H. Lichtman**[2,*]

[1]Departments of Medicine, Physiology, and Pathology & Cell Biology Columbia University Medical Center, New York, NY 10032, USA

[2]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

Abstract

Atherosclerosis is an arterial disease process characterized by the focal subendothelial accumulation of apolipoprotein B-lipoproteins, immune and vascular wall cells, and extracellular matrix. The lipoproteins acquire features of damage-associated molecular patterns and trigger first an innate immune response, dominated by monocyte-macrophages, and then an adaptive immune response. These inflammatory responses often become chronic and non-resolving, leading to arterial damage and thrombosis-induced organ infarction. The innate immune response is regulated at various stages, from hematopoiesis through monocyte changes and macrophage activation. The adaptive immune response is regulated primarily by mechanisms that affect the balance of regulatory vs. effector T cells. Mechanisms related to cellular cholesterol, phenotypic plasticity, metabolism, and aging play key roles in regulating these responses. Herein we review select topics that shed light on these processes and suggest new treatment strategies.

A Brief Overview of Atherogenesis and Atherosclerotic Plaque Progression

Atherogenesis is initiated by the entry and retention of apolipoprotein B-containing lipoproteins (apoB LPs) into the subendothelial space, or "intima," at regions of disturbed blood flow in medium-sized arteries (Williams and Tabas, 1995; Fogelstrand and Boren, 2012). The amount of apoB LP retention is determined by their concentration in the blood, age of the individual, metabolic state, and genetic and environmental factors. These considerations affect arterial wall biology, including variations in subendothelial proteoglycans that retain apoB LPs and factors that alter endothelial permeability. Initially, some of the LP lipid is internalized by resident CD11c^{+} myeloid cells, and experimental depletion of these cells suppresses the accumulation of foam cells and intracellular lipids within 5 days after cellular depletion (Paulson et al., 2010). Then, certain lipid and protein components of subendothelial apoB LPs, particularly after oxidative modification, take on properties of damage-associated molecular patterns (DAMPs) and thereby trigger an inflammatory response (Glass and Witztum, 2001; Lusis, 2000). The response activates

Correspondence: iat1@columbia.edu (I.T.), alichtman@bwh.harvard.edu (A.H.L.).

endothelial cells, which, together with flow-mediated changes in these cells (Jongstra-Bilen et al., 2006; Gimbrone, Jr. and Garcia-Cardena, 2013), promotes the entry into the intima of bone marrow-derived monocytes (Tacke et al., 2007; Swirski et al., 2016). The Ly6C^{hi} subpopulation of monocytes in the intima differentiate into macrophages, which, in progressing lesions, take on an inflammatory phenotype (Tacke et al., 2007; Swirski et al., 2007). In part as a result of the accumulation of inflammatory macrophages and dendritic cell activation, an inflammatory adaptive immune response develops involving primarily T helper-1 (Th1) T cells, but also Th17 and Th2 T cells and B cells, and there is a progressive decrease in regulatory T cells (Treg) (Witztum and Lichtman, 2014). Other immune cells, including neutrophils and platelet-neutrophil aggregates, innate immune cells, natural killer cells, mast cells, and eosinophils are present in human atheroma and have been shown to promote atherosclerosis via additional mechanisms in mouse models (Witztum and Lichtman, 2014). Accompanying this immune cell reaction is the accumulation of myofibroblasts in the intima that arise from medial smooth muscle cells and other sources and are referred to as vascular smooth muscle cells (VSMC) (Bennett et al., 2016). These cells are rich sources of extracellular matrix (ECM), which likely represents a "scar" response to inflammation and the ongoing vascular injury.

In a physiologic post-inflammatory response, macrophages and other inflammatory cells secrete molecules and carry out functions that dampen the inflammatory response and promote tissue repair (Serhan et al., 2007; Nathan and Ding, 2010). However, as will be explained later in this review, this so-called resolution response can go awry in the setting of atherosclerosis. Impaired resolution in atherosclerotic lesions leads to sustained, non-resolving, and maladaptive inflammation that promotes plaque progression and, in humans, triggers acute thrombo-occlusive cardiovascular events (Merched et al., 2008; Tabas, 2010; Viola and Soehnlein, 2015) (below). The pathological features of clinically dangerous plaques include large areas of necrosis and thinning of an overlying collagenous, or fibrous, cap. When a breach forms in the fibrous cap, the blood is exposed to thrombogenic material in the lesion, and acute occlusive thrombosis with tissue infarction can ensue (Virmani et al., 2002; Libby, 2013). However, acute thrombotic vascular events can also occur in the vicinity of more fibrous, non-necrotic plaques that are characterized by endothelial erosion (Libby, 2017). Studies in mice have suggested that this latter process is promoted by flow disturbance and neutrophil-mediated effects on endothelial cells (Franck et al., 2017).

In the sections that follow, we will review a selective subset of innate and adaptive immune processes that have recently come to light as affecting atherogenesis and/or plaque progression. The reader is referred to the reviews and original references cited above for the many important immune processes in atherosclerosis that are not included herein.

Changes in Monocyte Dynamics Contribute to Atherogenesis

The abundance of monocytes in the circulation, particularly those of the CD14^{++} subpopulation in humans and Ly6C^{hi} subpopulation in mice, is strongly correlated with atherosclerotic vascular disease in humans and with atherosclerotic lesion development in mice (Olivares et al., 1993; Murphy and Tall, 2016). In this context, recent studies have provided fascinating new insight into the regulatory mechanisms of monocytosis relevant to

atherosclerosis (Figure 1). The role of the sympathetic nervous system (SNS) has come to light as researchers sought to explain why atherosclerosis accelerates after myocardial infarction (MI). In the setting of an inflammatory response, Ly6C^{hi} monocytes initially give rise to macrophages on the inflammatory end of the inflammatory-resolution spectrum, and, as will be discussed below, these macrophages promote atherosclerosis progression (Swirski et al., 2007). A substantial portion of Ly6C^{hi} monocytes that contribute to atherosclerosis originate from the spleen, which becomes populated with bone marrow-derived hematopoietic stem and progenitor cells (HSPCs) and carries out extramedullary hematopoiesis (Robbins et al., 2012). In this context, mouse studies suggest that a key mechanism of post-MI atherosclerosis is SNS-mediated release of HSPCs from the bone marrow, leading to seeding of the spleen, elevated extramedullary hematopoiesis, and increased release of Ly6C^{hi} monocytes, which are the subset of monocytes that drive atherogenesis (Dutta et al., 2012). Further evidence suggests that other stress-related events that are known to be risk factors for atherosclerotic disease, such as psychosocial stress, may work through a similar mechanism (Heidt et al., 2014). How these concepts apply to human atherothrombotic vascular disease remains an important area for future study, particularly in view of uncertainties related to the functions of CD14^{++} monocytes in humans (Hilgendorf and Swirski, 2012).

Hypercholesterolemia also promotes monocytosis in mice, particularly Ly6C^{hi} monocytosis (Swirski et al., 2007), and genetically engineered defective cholesterol efflux in HSPCs can also exacerbate Ly6C^{hi} monocytosis (Murphy and Tall, 2016) (Figure 2). The mechanism responsible for cholesterol-induced monocytosis involves expansion of Lin$^-$ cKit$^+$ Sca1$^+$ (LSK) HSPCs in the marrow compartment (Murphy and Tall, 2016). Mechanistic studies revealed that cholesterol-mediated changes in the plasma membrane of HSPCs leads to elevated expression of the common β-subunit of the interleukin-3 (IL-3) receptor and granulocyte-monocyte colony stimulating factor (GM-CSF) receptor on the cell-surface and increased sensing of two key HSPCs growth factors, IL-3 and GM-CSF (Yvan-Charvet et al., 2010). There is also evidence that hypercholesterolemia, presumably by increasing the cellular content of cholesterol, decreases the expression of Rb, a tumor suppressor that limits HSPC proliferation, and increases the expression cyclins B1, D1, and E1 in HSPCs (Seijkens et al., 2014). Interestingly, when normocholesterolemic recipient mice are transplanted with bone marrow from either normocholesterolemic or hypercholesterolemic donor mice, the HSPCs of hypercholesterolemic donor origin showed increased proliferation 10 weeks later (Seijkens et al., 2014). These data suggest a long-lived, cell-intrinsic effect of hypercholesterolemia on HSPCs, perhaps due to epigenetic changes in the donor HSPCs. Moreover, when *Ldlr*$^{-/-}$ mice are transplanted with bone marrow cells from hypercholesterolemic versus normocholesterolemic mice and then fed a Western-type diet rich in cholesterol and saturated fats, the mice that had received that hypercholesterolemic bone marrow developed larger and more advanced lesions. This increase in atherosclerosis is accompanied by a higher number of lesional leukocytes derived from the hypercholesterolemic mouse bone marrow cells and overall increases in plaque macrophages, granulocytes, and T cells.

Other recent studies have taken advantage of the fact that leukocytosis in myeloproliferative diseases (MPD) is associated with atherothrombotic vascular disease (Murphy and Tall,

2016). For example, a loss-of-function polymorphism in the gene encoding a signaling adaptor protein called LNK (SH2B3) is associated with both MPD and atherosclerosis (McMullin et al., 2011; Deloukas et al., 2013). Moreover, somatic gain-of-function mutations in the JAK2 kinase are also associated with MPD and atherosclerotic disease (Viny and Levine, 2014). While processes related to neutrophils and platelets likely contribute to the mechanism of this association, there is also a link to monocytosis. In this context, genetic targeting of *Lnk* in Western diet-fed *Lnk$^{-/-}$Ldlr$^{-/-}$* has been found to cause hypercholesterolemia-dependent monocytosis, which is associated with increased amounts of the pro-atherogenic chemokine monocyte chemotactic protein-1 (MCP-1; CCL2) (Wang et al., 2016). Most importantly, these mice demonstrate increases in atherosclerotic lesion area, lesional macrophages, Ly6C^{hi} monocyte entry into lesions, and atherogenic platelet-monocyte aggregates (Wang et al., 2016). There is synergy between hypercholesterolemia and LNK deficiency, leading to further increases in IL-3-GM-CSF receptor signaling in bone marrow HSPCs. Further insight into some of these processes emerge from a study showing that Glut1-mediated glucose uptake by inflammatory myeloid cells promotes myeloproliferation in mice with either defective cholesterol efflux or myeloproliferative disorders (Gautier et al., 2013). In view of the link between glycolysis and inflammatory myeloid cell function (Van den Bossche et al., 2017) (below), the authors propose that Glut1-mediated glucose uptake provides the energy necessary for inflammatory myeloid cell proliferation.

Ly6C^{lo} monocytes derive from Ly6C^{hi} monocytes and serve an endothelial maintenance "patrolling" function in the circulation, but their roles in tissues has become increasingly unclear, and it is not even certain whether they differentiate into macrophages (Jakubzick et al., 2017). As such, the role of Ly6C^{lo} monocytes in atherosclerosis is poorly understood. In one study, investigators have tested the effect of targeting the nuclear receptor Nr4a1 (Nur77), which is required for the differentiation and survival of Ly6C^{lo} monocytes (Hanna et al., 2011). Two models of mouse atherosclerosis with genetic targeting of *Nr4a1*— Western diet-fed *Ldlr$^{-/-}$* mice lacking hematopoietic *Nr4a1* and *Apoe$^{-/-}$* mice with germline targeting of *Nr4a1*—demonstrate increased atherosclerosis, and this is associated with an increase in the proportion of lesional macrophages that have an inflammatory *vs.* resolving phenotype (Hanna et al., 2011). While one interpretation of this finding is that Ly6C^{lo} monocytes directly give rise to resolving macrophages that regulate the inflammatory response, another possible interpretation needs to be considered in view of the uncertainty as to whether Ly6C^{lo} monocytes differentiate into macrophages and based on the results of a study examining post-myocardial infarction (MI) repair (Hilgendorf et al., 2014). Ly6C^{hi} monocytes infiltrate the heart immediately post-MI and then, during the repair phase, give rise to Ly6C^{lo} macrophages with resolving properties. However, in mice lacking hematopoietic *Nr4a1*, infiltrating Ly6C^{hi} monocytes differentiate into highly inflammatory macrophages that are unable to carry out tissue repair. If applicable to atherosclerosis, the data from the Nr4a1-deficient mouse study above may suggest that Ly6^{hi} monocytes give rise to reparative macrophages in atherosclerotic lesions in a *Nr4a1*-dependent manner. Consistent with this idea, a recent study shows that Ly6C^{hi} monocytes are the source of resolving macrophages during atherosclerotic plaque regression (Rahman et al., 2017).

Inflammatory Macrophages Drive Atherosclerosis Progression

Macrophage functions can vary widely depending on a number of interacting variables and factors, including local environment ("tissue niche") (Gosselin et al., 2014; Lavin et al., 2014); intracellular energy metabolism (Van den Bossche et al., 2017); gut microbiota metabolites (Wang et al., 2011); and genetic and epigenetic factors, including non-coding RNAs (Erbilgin et al., 2013; Chen et al., 2015; Wu et al., 2016; Amit et al., 2016; Phan et al., 2017; Aryal et al., 2014). These factors program macrophages for functions on a spectrum from inflammatory and host defense to resolution and repair. In general, inflammatory macrophages carry out processes that promote atherosclerosis progression, whereas resolving macrophages carry out functions that can suppress plaque progression and promote plaque regression (Peled and Fisher, 2014) (Figure 1). By secreting cytokines, proteases, and other factors, inflammatory macrophages increase the cellular expansion of lesions and cause changes in plaque morphology that can trigger plaque rupture and acute lumenal thrombosis. Two key changes in plaque morphology promoted by inflammatory macrophages are plaque necrosis and thinning of a protective collagenous scar (fibrous cap). Conversely, resolving macrophages carry out functions that are associated with plaque stabilization. These functions include clearing dead cells (efferocytosis), which stabilize plaques by preventing post-apoptotic cellular necrosis (below); secreting collagen that can form a protective scar over the lesion; and producing proteins and lipids that quell inflammation and promote tissue repair.

Molecular profiling of lesional macrophages at various stages of lesion progression and regression has demonstrated heterogeneity suggestive of these different functions (Peled and Fisher, 2014). One such study using immunohistochemistry and RNA profiling shows that CD68$^+$ macrophages at both ends of the inflammation-resolution spectrum accumulate as atherosclerotic lesions develop (Stoger et al., 2012). The relative proportion of macrophages with different phenotypic markers vary depending on plaque region. For example, macrophages on the inflammatory end of the spectrum are enriched in regions of the plaque that are prone to rupture. In this regard, inflammatory macrophages can secrete matrix metalloproteinases, *e.g.*, MMP2 and MMP9, which may contribute to plaque rupture, and another study shows that MMPs co-localize with inflammatory macrophages in advanced plaques (Huang et al., 2012). Conversely, plaques that appear more stable based on thicker fibrous caps and smaller areas of plaque necrosis are found to be enriched in macrophages towards the resolution end of the spectrum., which is consistent with the aforementioned plaque-stabilizing functions of resolving macrophages. In a similar vein, regressing plaques, which can be modeled in mouse atherosclerosis by abruptly and markedly lowering plasma cholesterol, show a shift toward the resolution phenotype (Trogan et al., 2006).

Important gaps remain in our understanding of the mechanisms leading to macrophage heterogeneity in various stages of atherosclerosis progression and regression and at different sites within the plaques themselves. A key question is the relative contribution of changes in the types of infiltrating monocytes (above section) versus changes in pre-existing lesional monocytes or macrophages, such as by proliferation of inflammatory macrophages and phenotypic switching. Macrophage proliferation occurs in atherosclerosis, particularly in advanced plaques (Sakai et al., 2000; Robbins et al., 2013), but the mechanisms and

consequences of lesional macrophage proliferation remain largely unknown. In one study, investigators have studied the effect of heterozygous deficiency of a *Zfp148*, zinc-finger protein that suppresses the checkpoint protein P53, in hematopoietic cells in atheroprone *Apoe*$^{-/-}$ mice (Sayin et al., 2014). These mice demonstrate a decrease in lesional macrophage proliferation, and this was associated with decreases in markers of inflammation and overall lesion area in a P53-dependent manner. Several mechanisms driving macrophage proliferation in atherosclerosis have been proposed, including activation of scavenger receptor class A signaling, activation of the GTPase Ras, and loss of function of the cyclin-dependent kinase Inhibitor 2A (*CDKN2A*), which encodes two cell cycle inhibitors and is linked to a susceptibility locus for atherothrombotic disease in humans (Sakai et al., 1996; Senokuchi et al., 2005; Kuo et al., 2011). Regarding the Ras hypothesis, a recent study shows that delivery of an HMG-CoA reductase inhibitor (statin), which blocks Ras activation, to lesional macrophages decreased lesional macrophage proliferation and plaque inflammation (Tang et al., 2015).

Much of the effort in understanding phenotypic modulation of lesional macrophages has focused on environmental cues in lesions that can affect macrophage phenotype. For example, accumulation of cholesterol in these cells can trigger inflammatory signaling pathways, presumably by effects on the plasma membrane that activate inflammatory receptors, *e.g.*, toll-like receptors (TLRs) (Westerterp et al., 2014) (Figure 2). Important new work has suggested that this cholesterol-dependent process in macrophages can be exacerbated in the setting of diabetes by the incorporation of endogenously synthesized fatty acids in cholesterol-rich domains of the plasma membrane (Wei et al., 2016). Moreover, cholesterol accumulation in macrophages activates the inflammasome, leading to increased production of pro-atherogenic interleukin 1β. While cholesterol crystals have been implicated in this process (Duewell et al., 2010; Sheedy et al., 2013), other mechanisms likely contribute as well (Hoseini et al., 2017).

Some of the work in this area has been linked to epigenetic changes at early stages of monocyte-to-macrophage differentiation. For example, exposure of monocytes to oxidized LDL, a modified form of LDL that accumulates in atherosclerotic plaques, "primed" the cells for a subsequent increase in their inflammatory response to TLR2 and TLR4 activators (Bekkering et al., 2014). This response is associated with an epigenetic histone mark of active transcription (trimethylation of H3K4) in the promoters of several of the up-regulated inflammatory genes, and the priming response could be blocked by a methyltransferase inhibitor. A similar finding has been found by comparing monocytes from subjects with symptomatic *vs.* asymptomatic carotid atherosclerosis (Bekkering et al., 2016). Another study shows that HDAC3 inhibitors increase the expression of ABCA1 and ABCG1 in cultured macrophages and that myeloid deletion of HDAC3 in Western diet-fed *Ldlr*$^{-/-}$ mice suppresses atherosclerosis and promotes a more resolving phenotype in lesional macrophages (Hoeksema et al., 2014). The inhibitors used in these studies cause broad changes in histone marks throughout the genome, and thus future studies will be needed to define specific epigenetic changes that contribute to pro-atherogenic responses in lesional macrophages.

An Increase in the Ratio of Effector T Cells to Regulatory T Cells Promotes Atherosclerosis

As discussed above for macrophages, T cell functional phenotypes can change in response to environmental clues, thereby altering their relative ability to function as regulatory vs. inflammatory cells. The concept that Treg cells can respond to inflammatory mediators in local tissue environments and take on some of the phenotypic properties of effector T cells (Teff) in those sites is well established. In some cases, Teff-like Treg cells retain their suppressive functions, but in other cases such as in autoimmune disease models, Treg cells assume the inflammatory and pathogenic roles of their Teff cell cousins (Sawant and Vignali, 2014). There is a selective decrease in Treg cells compared with Teff cells during atherosclerotic lesion development (Maganto-Garcia et al., 2011), and this could be attributed to phenotypic plasticity, as well as Treg vs. Teff cell differences in migration into lesions or susceptibility to death (Figure 3). FoxP3 and T-bet are the lineage-defining transcription factors required differentiation and function of most CD4$^+$ Treg or Th1 cells, respectively, and plasticity of these subsets is tied to changes in their expression. Recent studies in mouse models provide evidence of Treg cell plasticity driven by the atherosclerotic and hypercholesterolemic environments, resulting in cells with impaired regulatory or enhanced inflammatory functions. In one study, the accumulation of CD4$^+$CCR5$^+$IFNγ^+FoxP3$^+$T-bet$^+$ cells (referred to as Th1-Treg cells) were shown to accumulate in atherosclerotic lesions of *Apoe$^{-/-}$* mice. Adoptive transfer studies of CD4$^+$ T cells carrying FoxP3 fate mapping reporter genes into *Apoe$^{-/-}$* mice indicate the Th1-Treg cells are derived from former Treg but not naïve T cells (Butcher et al., 2016). Furthermore, the conversion to Th1-Treg cells does not occur in non-atherosclerotic C57BL/6 mice. The Th1-Treg cells show impaired regulatory functions *in vitro* and reduced expression of several genes linked to Treg cell immunosuppressive function, and they fail to attenuate plaque burden *in vivo*. Data from another independent study shows that up to 40% of the CD4$^+$ T cells in atherosclerotic aorta of *Apoe$^{-/-}$* mice have a CCR5$^+$ FoxP3$^+$T-bet$^+$ phenotype and produce abundant IFN-γ and TNFα (Li et al., 2016). These cells, named FoxP3$^+$CCR5$^+$CD25$^-$ Teff cells by the investigators, do not suppress Teff cell proliferation and promote atherosclerosis in adoptive transfer experiments. Whether FoxP3$^+$CCR5$^+$ Teff cells identified in that study are derived from plastic conventional Treg cells remains unknown. However, because they are only found in aorta and peri-aortic lymph nodes and not in spleen, it is possible that they are derived from plaque Treg cells in response to factors in the lesional environment. Interestingly, studies in other lineage-specific T-bet deletion and FOXP3-reporter models, without hypercholesterolemia or atherosclerosis have demonstrated that T-bet expressing Treg cells are immunosuppressive and required to prevent autoimmunity (Levine et al., 2017; Yu et al., 2015). This apparent contradiction in the observed functions of T-bet expressing Treg cells may indicate that the development of IFN-γ-expressing inflammatory T-bet$^+$ Treg cells is linked to the unique conditions of systemic hypercholesterolemia and/or the plaque micro-environment.

Many studies of helper T cell subsets in human autoimmune conditions and mouse models of these diseases reinforce the concept that Teff cells are plastic and can differentiate within inflamed issues toward more pathogenic phenotypes during disease progression. For

example, under the influence of innate inflammatory cytokines, Th17 cells can be changed from barrier protective effector T cells that secrete IL-17A and IL-10 into pathogenic effectors that secrete IL-17A, IL-22, and IFN-γ (Burkett et al., 2015)(Burkett et al., 2015). This Th17 cell conversion into pathogenic effector cells is promoted by IL-1, IL-6, and IL-23, all of which are known to be expressed in atherosclerotic lesions. The contribution of IL-17 or Th17 cells to atherosclerotic lesion development remains uncertain based on mouse studies, but dual IL-17- and IFN-γ-producing T cells are present in atherosclerotic human coronary arteries at a higher frequency than in the blood (Eid et al., 2009). IL-23 receptor expression on Th17 cells plays a non-redundant role in their pathogenic conversion, and Notch signaling via the DNA binding protein RBPJ is required for induction of IL-23R expression by Th17 cells (Meyer zu et al., 2016). In this context, a recent report shows that abrogation of RBPJ-dependent Notch signaling reduces atherosclerosis in $Apoe^{-/-}$ mice, although the importance of T cell IL-23R expression in that study was not addressed (Nus et al., 2016).

Although T cells do not accumulate large amounts of cholesterol in the setting of hypercholesterolemia as do macrophages, exposure to oxidized LDL and perturbations of T cell intrinsic cholesterol metabolism have profound effects on T cell functions and are implicated in T cell phenotypic modulation (Figure 2). The liver has been long recognized to be an important tissue site for the induction of peripheral T cell tolerance. Given the central role of the liver in handling dietary cholesterol and synthesis of lipoproteins, it is interesting to consider how hepatic T cells respond to changes in cholesterol homeostasis. Hypercholesterolemia in Western diet-fed $Ldlr^{-/-}$ is reported to increase the numbers of intrahepatic Treg cells and expression of TGF-β, a cytokine that both drives Treg cell differentiation and is a principal immunosuppressive mediator secreted by Treg cells(Mailer et al., 2017). Adoptive transfer experiments show that these hepatic Treg cells home to atherosclerotic aortas. Furthermore, hypercholesterolemia induces either Th1 or Th17 cell differentiation of hepatic T cells depending on the presence or absence of LDL receptors. These data suggest that systemic changes in lipoprotein metabolism and dietary cholesterol may change the ratios of Treg and Th cell subsets generated in the liver and then enter plaques.

The mechanisms by which hypercholesterolemia may influence Treg or Teff cell differentiation likely involve effects on the antigen presenting DCs, which are found in atherosclerosis prone regions of arteries before lesion development and readily take up oxidized-LDL (Paulson et al., 2010). DCs maintain their antigen-presenting functions after becoming foam cells (Packard et al., 2008), and respond to cholesterol accumulation by increasing production of cytokines that drive Th cell subset differentiation (Westerterp et al., 2017). However, there also appear to be direct effects of cholesterol on T cells. For example, T cells from mice deficient in the cholesterol transporter ABCG1 show increased proliferative responses to TCR stimulation, and T cell-selective deletion of the $Abcg1$ gene results in an increase in Treg cell differentiation from naïve T cells (Armstrong et al., 2010). T cell- or Treg-cell-restricted ABCG1 deficiency in $Ldlr^{-/-}$ mice results in more Treg, less Teff cells, and less atherosclerosis compared to control mice (Cheng et al., 2016). Interestingly, the ABCG1-deficient T cells accumulate more cholesterol in lysosomes than control T cells, which lead to inhibition of mTOR-mediated STAT5 signaling. These

changes in energy metabolism favor Treg over Teff cell phenotypes, as discussed in the next section. T cell activation requires the assembly of antigen receptor and other signaling molecules in cholesterol-rich lipid rafts, which is a prerequisite to the formation of immune synapses with antigen presenting cells. Therefore, alterations in cellular cholesterol may influence T cell activation because of changes in lipid raft formation. This was shown to be the case for CD8+ effector T cells in mice in which cellular unesterified cholesterol was elevated by interventions that block cholesterol ester formation (Yang et al., 2016). CD8+ T cell plasma membrane cholesterol was increased in these mice, and anti-tumor effector functions of the CD8+ T cells were increased. Thus, the net immunologic impact of changes in T cell cholesterol appear to vary depending on the intracellular compartment affected and on the T cell subset.

In two of the previously discussed studies, exposure of T cells to excess cholesterol, either through systemic hypercholesterolemia (Mailer et al., 2017) or by impairing cholesterol efflux by deleting ABCG1 (Cheng et al., 2016), was shown to favor Treg over Teff cell differentiation. This may seem surprising, given the atheroprotective effect of Treg cells and the pro-atherogenic and pro-inflammatory effects of excess cholesterol and Teff cells. Perhaps the direct proinflammatory effects of hypercholesterolemia on macrophages and DCs overwhelms those on Treg cell development. Another possibility is that Treg cells generated in response to the cholesterol stimuli differentiate into Th1-like cells under hypercholesteremic conditions, as discussed earlier. In general, induction of regulatory mechanisms concurrently with inflammatory responses is commonplace in the immune system, which must strike a fine balance between anti-microbial defense and protection of host tissues. This balance and the timing of the counteracting mechanism achieves defense against pathogens followed by resolution and return to homeostasis, thereby limiting collateral damage to healthy tissues and avoiding autoimmunity. In atherosclerosis, an emerging theme is that the balance is pathologically tipped toward the inflammatory side, and the regulatory mechanisms fail to move the scales to achieve resolution.

Energy Metabolism Affects Immune Cell Function in Atherosclerosis

An area of increasing interest is how changes in macrophage metabolism influence their phenotype. Studies using cultured macrophages treated with either lipopolysaccharide (LPS) and interferon γ (IFNγ) to model inflammatory macrophages or with IL-4 to model of so-called alternatively activated macrophages have revealed certain preferences for metabolic pathways, but these associations are more complex than generally portrayed (Van den Bossche et al., 2017). For example, fatty acid oxidation (FAO) and oxidative phosphorylation are needed to support the phenotype of alternatively activated macrophages, but FAO is also involved in inflammasome activation in inflammatory macrophages. Conversely, while glycolytic metabolism has been associated with energy metabolism in inflammatory macrophages, glycolysis is also used by alternatively activated macrophages to fuel the tricarboxylic acid cycle for mitochondrial respiration. The role of metabolic shifts in determining the phenotype of macrophages in atherosclerotic lesions is not yet well-characterized, but a few recent studies have shed light on this interesting topic. As one example, inflammatory macrophages are found to have increased expression of a particular microRNA, miR-33, and when miR-33 is silenced in cultured macrophages, the cells show

markers of alternative activation associated with increased expression of AMP kinase and FAO genes, elevated mitochondrial respiration, and decreased glycolysis (Ouimet et al., 2015). *In vivo*, treatment of Western diet-fed *Ldlr*^{-/-} mice with an anti-miR-33 leads to a reduction in both plaque size and complexity and a change in lesional macrophage gene expression profile suggestive of alternative activation. Moreover, these changes are accompanied by an increase in athero-protective regulatory T cells in lesions, suggesting a link between immunometabolism in macrophages and innate-adaptive immunity crosstalk in the setting of atherosclerosis. As another example, investigators reported elevated glucose uptake and glycolytic flux in monocytes and macrophages from patients with coronary artery disease (CAD) compared with control subjects (Shirai et al., 2016). *In vitro* experiments show that this metabolic phenotype leads to activation of a pathway involving mitochondrial oxidative stress (mitoOS) and pyruvate kinase M2-mediated STAT3 activation, resulting in increased production of pro-atherogenic IL-6 and IL-1β. Other studies have suggested additional links between mitoOS and human CAD (Paneni et al., 2017), and mitoOS in macrophages increases plaque progression, activates macrophage NF-κB, and promotes the formation of potentially atherogenic neutrophil extracellular traps (NETs) in mouse models of atherosclerosis (Wang et al., 2014; Wang et al., 2017).

In a follow-up to the glycolytic flux study above, the investigators found that the elevated glycolytic flux in macrophages from CAD subjects leads to excess mitochondrial pyruvate, which drives BMP4- and IRF1-dependent expression of the T cell inhibitory protein PD-L1 (Watanabe et al., 2017). The high PD-L1 expression on these macrophages was shown to block T cell activation, and the investigators correlated this with impaired anti-varicella zoster virus immunity in the CAD patients. Interestingly, one mechanism of the de-activating effect of PD-1 on T cell activation may involve PD-1-mediated suppression of glycolysis in the T cells themselves, as has been shown recently for CD8+ T cells (Bengsch et al., 2016). As with the miR-33 mentioned above, glycolysis-induced PD-L1 in macrophages represents another example of metabolic changes in macrophages linked to regulation of T cells, except in this case glycolysis-driven macrophage inflammation down-regulates T cell activation. This T cell-suppressive process represents another example of a counter-regulatory pathway that is induced concurrently with induction of pro-inflammatory pathways. PD-L1 induction does not prevent lesion development, but likely provides a beneficial effect by limiting lesion growth and inflammation. Consistent with this concept is the finding that deficiency of PD-1 or PD-L1 in high fat-diet-fed *Ldlr*^{-/-} mice enhances lesion development and plaque inflammation compared with baseline lesion development in wild-type mice that occurs despite the presence of PD-L1 (Lichtman, 2012). Similarly, a recent study shows that hypercholesterolemia induces PD-L1 expression on marginal zone B cells, which inhibits pro-atherogenic T follicular helper cell responses (Nus et al., 2017).

Another environmental factor in advanced atherosclerotic lesions that can affect macrophage metabolism is hypoxia. Hypoxic conditions exist in atherosclerotic plaques, leading to increased expression of hypoxia-inducible factor-1α (HIF-1α) (Marsch et al., 2013). A recent study finds that hypoxia markedly increases glycolysis in cultured macrophages exposed to inflammatory cytokines or oxidized LDL. This effect is associated with a HIF-1α-dependent increase in mRNAs encoding glycolysis-promoting enzymes, notably hexokinase II (*Hk2*) and the 6-phosphofructo-2-kinase (*Pfkfb3*), and with an amplified

production of inflammatory cytokines (Tawakol et al., 2015). In Western diet-fed *Apoe*^{-/-} mice, inhibition or silencing of either *Pfkfb3* or *Hif1a* attenuates lesional glycolysis and decreases the expression of inflammatory cytokines, although the net effect on lesion development was not reported. While this study suggests that HIF-1α can be pro-inflammatory, HIF-1α in CD11c-expressing cells may have the opposite effect, as CD11c-cre-mediated deletion of *Hif1a* in *Ldlr*^{-/-} mice results in increased lesion development and more T cells in the plaques (Chaudhari et al., 2015). The HIF-1α-deficient APCs express less STAT3 and more IL-12, which is associated with increased Th1 cell differentiation, and lentivirus-induced overexpression of STAT3 in bone marrow cells reverses the effects of the *Hif1a* deletion *Lyz2-cre*-mediated *Hif1a* deletion does not have the same effects on lesion development or T cells, indicating that HIF-1α deficiency in dendritic cells is the likely cause of the pro-atherogenic phenotype.

As discussed for macrophages (above), changes in energy metabolism in T cells are also linked to functional phenotypes, and these metabolic-functional T cell states may synergize with proatherogenic or resolving functions of monocytes and macrophages within atherosclerotic lesions or systemically. In this regard, two differences between T cells and macrophages that should be kept in mind are (a) the requirement for rapid proliferative expansion to generate effector T cells from naïve or resting memory cells; and (b) the greater diversity of specialized functional phenotypes of T cells. These features are reflected in many different metabolic states in T cells, depending on the stage of the T cell response and the functional subset of the T cell (MacIver et al., 2013). Resting naïve T cells use the TCA cycle to oxidize glucose-derived pyruvate, lipids and amino acids, and efficiently generate ATP. Antigen-induced clonal proliferation requires a shift to aerobic glycolysis with reduced lipid oxidation and increased lipid synthesis. The resting memory T cells that survive at the end of a T cell response go back to efficient energy generation, but mainly by lipid oxidation. Furthermore, there are distinct metabolic phenotypes in different T cell subsets. Effector helper T cells, including Th1, Th2, and Th17 cells require mTOR and aerobic glycolysis for differentiation. However, whereas Th1 and Th17 cells depend on active mTORC1 activity, Th2 cells depend on mTORC2. In contrast, Treg cell generation is regulated by AMP-activated protein kinase (AMPK) and relies on lipid oxidation through the TCA cycle. Given the opposing roles of Th effector cells and Treg cells on atherosclerosis, summarized earlier, and the possible different influences of Th cell subsets, the impact of systemic modulators of cellular metabolism associated with atherosclerotic disease and the local metabolic environment of the plaque are likely to affect how T cells contribute to lesion progression.

Of relevance to how the local plaque environment may influence T cell metabolism and function, a recently published study has shown that effector memory T cells (Tem), the functional phenotype of most T cells found in plaques, responds to hypoxia by increased HIF-1α expression, which in turn increases proliferation, viability, and cytotoxicity (Xu et al., 2016). This is not the case for naïve or central memory T cells, and, as discussed earlier, is in contrast to the finding of increased T cell inflammation in plaques in Western diet-fed *Ldlr*^{-/-} mice with DC-restricted deletion of *Hif1a* (Chaudhari et al., 2015). GAPDH has been shown to block HIF-1α translation, and the increased glycolytic activity in Tem reduced GAPDH, resulting in elevated HIF1α expression. A recent study has shown that FoxP3, the

defining Treg cell transcription factor, is responsible for maintaining a metabolic state in which glycolysis is repressed and oxidative phosphorylation and nicotinamide adenine dinucleotide oxidation are increased. This state allows Treg cells to function in low-glucose, lactate-rich environments that impair effector T cell function and proliferation (Angelin et al., 2017). The likely influence of the plaque environment on T cell metabolic states and function have not yet been experimentally established.

Aging Affects Immune Cell Function in Atherosclerosis

Aging is major risk factor for atherosclerotic disease (Lakatta and Levy, 2003). One of the consequences of aging is an accumulation of somatic mutations in hematopoietic stem cells (HSC), which has been linked to increased clonal hematopoiesis and cardiovascular disease (Jaiswal et al., 2014; Jaiswal et al., 2017) (Figure 2). One of the genes affected by age-related HSC somatic mutations is *TET2*, which encodes an epigenetic modifier enzyme that can also repress inflammatory gene expression in human and mouse macrophages and DCs by recruiting histone deacetylases (HDAC) (Zhang et al., 2015). Among the genes repressed by TET2 include IL-6 and several chemokines. In Western diet-fed *Ldlr*$^{-/-}$ mice, partial bone marrow reconstitution with TET2-deficient hematopoietic cells leads to clonal expansion of these cells, with a myeloid bias, and increased atherosclerosis with an enrichment of *Tet2*$^{-/-}$ lesional macrophages (Fuster et al., 2017; Jaiswal et al., 2017). The pro-atherogenic effect TET2 deficiency in mice has been ascribed to inflammasome activation (Fuster et al., 2017). Mechanistic studies have shown that the ability of TET2 to suppress the inflammasome does not depend on its canonical activity of oxidizing 5-methylcytosine but rather on HDAC-mediated suppression of *Il1b* and inflammasome component genes. Because the *in vivo* causation study in this report used a chemical inhibitor of the inflammasome, the relative contribution of the inflammasome to the pro-atherogenic effect of TET2 deficiency will need to be confirmed using genetic approaches. Other areas for future studies include relevance to human lesions in subjects with somatic *TET2* mutations and the impact of somatic mutations on DC function and T cell responses related to atherosclerosis. Importantly, one of the studies cited above has shown strong associations between coronary artery disease and age-related somatic mutations in a number of genes that cause clonal hematopoiesis of indeterminate potential (CHIP), including *TET2* but also *DNMT3A*, *ASXL1*, and *JAK2* (Jaiswal et al., 2017). These findings have potentially important implications related to risk stratification and possibly new and/or more tailored treatment strategies.

Aging is also associated with the accumulation of so-called senescent cells that, in chronic diseases of aging, can contribute to a maladaptive inflammatory response via secretion of inflammation-inducing factors. Advanced atherosclerotic lesions contain cells with markers of senescence, including senescence-associated β-galactosidase, p16^{Ink4a}, p53, p21, and shortened telomeres (Minamino and Komuro, 2007). While indirect causation studies in mice originally suggested a pro-atherogenic role of senescent lesional cells, genetic association studies in mice and humans have called into question this conclusion (Schierwagen et al., 2017). However, a more definitive causation study has been recently conducted using Western diet-fed *Ldlr*$^{-/-}$ mice in which lesional macrophages, VSMCs, and endothelial cells expressing p16^{Ink} were genetically eliminated (Childs et al., 2016). Compared with the control cohort, the engineered mice had smaller lesion size and

decreased lesion complexity without changes in pro-atherogenic systemic factors. In particular, the mice have a marked decrease in cholesterol ester-laden macrophages (foam cells) with markers of senescence, and this is accompanied by decreases in inflammatory cytokines and matrix metalloproteinases and an increase in fibrous cap thickness. In addition to the role of senescent cells in advanced plaque progression suggested by this study, an interesting observation is that senescent-appearing foam cell macrophages also accumulate in very early lesions and initiated processes that promote early atherogenesis.

Resolution of Inflammation is Impaired in Atherosclerosis and Contributes to Plaque Progression

In normal physiology, the inflammatory response is directly linked to a resolution phase that repairs collateral damage and restores tissue homeostasis (Serhan et al., 2007; Nathan and Ding, 2010). Resolution is mediated by (a) endogenous lipids that are generated during inflammation, including lipoxins, resolvins, protectins, and maresins, called specialized pro-resolving mediators (SPMs) (Serhan, 2010); and (b) proteins, such as IL-10, TGFβ, and annexin A1 (Perretti and D'Acquisto, 2009). By activating specific cell-surface receptors, these mediators block inflammatory cell influx and promote their egress; clear pathogens, cellular debris, inflammatory cytokines, and apoptotic cells (efferocytosis); and repair tissue damage (Serhan, 2010; Perretti and D'Acquisto, 2009). In chronic diseases in which the inciting pathologic process is persistent, resolution becomes defective owing to impaired synthesis or increased degradation of resolution mediators and to decreased resolution mediator action on cells that effect the repair process (Serhan, 2010). This defect leads to an amplification cycle of continual tissue injury and DAMP-mediated inflammation and, in the case of atherosclerosis, plays a key role in the clinical progression of plaques (Merched et al., 2008; Tabas, 2010; Viola and Soehnlein, 2015). In this context, several key features of clinically dangerous advanced plaques are characteristic of impaired resolution, including defective efferocytosis, plaque necrosis, and DAMP-mediated inflammation; thinning of the fibrous cap; and oxidative stress. Moreover, the ratio of resolving:inflammatory lipid mediators is markedly lower in advanced murine and human plaques compared with earlier lesions (Fredman et al., 2016; Viola et al., 2016). The therapeutic potential of enhancing resolution in chronic diseases like atherosclerosis could be substantial in that SPMs, unlike anti-inflammatory drugs, suppress inflammation and promote tissue repair in a manner that is less likely to compromise host defense than drugs directly targeting the inflammatory response (Serhan et al., 2007; Tabas and Glass, 2013) (below).

A lesional macrophage resolution process that has received particular attention in the field of atherosclerosis is efferocytosis (Tabas, 2005; Schrijvers et al., 2007; Linton et al., 2016; Van Vre et al., 2012). Efferocytosis is mediated through phagocyte receptors, apoptotic cell ligands, bridging proteins, and chemoattractants. It is normally a high-capacity and efficient process, but when it goes awry, tissue necrosis and subsequent DAMP-mediated inflammation occur (Henson et al., 2001; Camenisch et al., 1999). A critically important example of this principle is advanced atherosclerosis (Geng and Libby, 1995; Schrijvers et al., 2005; Otsuka et al., 2015). Clinically dangerous human coronary plaques show evidence of defective efferocytosis, *i.e.*, abundant uncleared dead cells, and this defect correlates with

two key features of these plaques—necrosis and inflammation. Causation is suggested by studies using genetically altered mice. For example, when efferocytosis is compromised through gene targeting of effector molecules, there is an increase in uncleared apoptotic cells, inflammation, and plaque necrosis (Yancey et al., 2010; Tao et al., 2015; Cai et al., 2017).

The unresolved mechanism of defective efferocytosis in advanced plaques represents a major gap in this field. In theory, defects could occur in processes occurring during apoptosis that normally ensure proper recognition or uptake by lesional phagocytes or in the phagocytes themselves. As an example of the former scenario, a recent study has presented evidence that lesional apoptotic macrophages, under the influence of inflammatory signaling, continue to express a "don't-eat-me" cell-surface protein called CD47 that is normally extinguished when cells die (Kojima et al., 2016). CD47, by interacting with a molecule on phagocytes called SIRPα, suppresses cell internalization by the phagocyte, and thus its persistence on lesional dead cells contributes to defective efferocytosis. With regard to phagocyte defects, there is evidence that lesional hypoxia may lead to decreased expression of a key efferocytosis receptor on macrophages called MerTK and thereby compromise the ability of lesional macrophages to recognize apoptotic cells (Marsch et al., 2014). Another mechanism of defective efferocytosis related to MerTK is ADAM17-mediated MerTK cleavage. There is evidence that MerTK cleavage occurs in advanced, necrotic human coronary plaques (Garbin et al., 2013; Cai et al., 2017), and Western diet-fed $Ldlr^{-/-}$ mice expressing an engineered non-cleavable mutant of MerTK show improved lesional efferocytosis and decreased plaque necrosis compared with mice expressing wild-type MerTK (Cai et al., 2017). Interestingly, these lesions also have additional signs of improved resolution, such as thicker fibrous caps, and they have an increase in the ratio of resolving:inflammatory lipid mediators, which is consistent with a prior examining holo-MerTK deficiency on plaque inflammation (Ait-Oufella et al., 2008). Mechanistic studies reveal a MerTK signaling pathway in macrophages that promotes the biosynthesis of resolving lipid mediators at the expense of inflammatory lipid mediators (Cai et al., 2016). These studies suggest an amplification loop in which resolving mediators promote efferocytosis, and then efferocytosis promotes the synthesis of resolving mediators. Thus, therapeutic strategies that could convert the pro-atherogenic cycle of defective efferocytosis, *i.e.*, impaired efferocytosis exacerbating DAMP-mediated inflammation, to this beneficial feedback cycle might be particularly effective at preventing advanced plaque progression.

Considering the intricate relationships between innate and adaptive effectors in chronic inflammatory processes, the macrophage contributions to defective resolution in plaques are likely to enhance and be aggravated by effector T cell responses. Conversely, pro-resolving interventions would be expected to engage regulatory T cells. There is evidence that Treg cells increase during the resolution phase of innate inflammatory processes in mouse models and help shape the resolution process. For example, after bacterial and worm infections in fate mapping reporter mice, Th17 cells have been shown to transdifferentiate into FoxP3 Treg cells as inflammation resolved (Gagliani et al., 2015). In a lung model of self-limited allergic inflammation, the SPM maresin 1 has been shown to induce Treg cells, which then inhibited type 2 innate lymphoid cells. A recent *in vitro* study has shown that SPMs can block differentiation of naïve human T cells to Th1 and Th17 cell effectors, block Teff cell

inflammatory cytokine production including IFN-γ, and induce Treg cells (Chiurchiu et al., 2016). These *in vitro* findings are supported by studies in *Elovl2*$^{-/-}$ mice, which are defective in SPM production and shown to have more Th1 and Th17 cells in lymphoid tissues and less Treg cells than control mice. Moreover, the fraction of aortic T cells that are FoxP3^{+} is increased in mice with enhanced plaque efferocytosis due to cleavage-resistant MerTK, described above (Cai et al., 2017). These initial reports set the stage for future studies that will further define T cell contributions to the resolution state and determine how these contributions may be leveraged by pro-resolving therapeutics in atherosclerosis.

Summary and Therapeutic Considerations

Our understanding of the ways innate and adaptive immunity influence atherosclerotic disease has continued to evolve along with emerging areas in immunology research. In this review, we have emphasized selected themes, including the dynamics and heterogeneity of macrophage and T cell phenotypes, the impact of cellular and systemic metabolic sates on immune cell phenotypes, and the ways macrophage and T cell functions impact the balance between progression and resolution of plaque inflammation. These emerging areas of investigation have raised new questions, but they have also validated efforts at modifying existing targeted therapeutic approaches and developing new strategies. Viewing the pathogenesis of atherosclerosis as an immunological reaction to retained and modified apoB LPs in the arterial wall emphasizes the fundamental importance and success of plasma apoB LP-lowering therapies, including statins, ezetimibe, and now PCSK9 inhibitors (Boren and Williams, 2016). By lowering the concentration of apoB LPs in the blood, which then lowers their infiltration into developing lesions, these therapies decrease the burden of DAMPs and antigens that drive innate and adaptive immune responses in the artery. Moreover, as discussed above, hypercholesterolemia can promote atherogenic activation of circulating immune cells and their precursors even before they enter developing lesions. In theory, if cholesterol-rich apoB LPs were lowered below a certain threshold concentration, this alone would halt plaque progression and likely induce regression. However, this potential has not been met due to a variety of issues, with the counteracting effects of obesity-induced insulin resistance playing a major role. Insulin resistance is a potent risk factor for atherosclerotic heart disease owing to its links to dyslipidemia and inflammation and to the effects of high insulin and glucose on atherosclerotic lesional cells (Razani et al., 2008; Bornfeldt and Tabas, 2011). Indeed, largely due to the epidemic of obesity and insulin resistance, atherosclerotic vascular disease remains the leading cause of death in the industrialized world, and its prevalence is expected to rise over the next 20 years (Go et al., 2013; Behn and Ur, 2006; American Heart Association and RTI International, 2015). In this regard, a topic of great interest moving forward is how insulin resistance might affect the immune processes discussed in this review and thereby exacerbate atherosclerosis (Han et al., 2006; Kanter et al., 2012; Nagareddy et al., 2013; Willecke et al., 2015). Moreover, as the number of patients being treated with PCSK9 inhibitors increases, we may have an opportunity to learn how lowering cholesterol below the concentrations achieved with statins alters immune parameters of atherosclerosis, even in the face of the obesity epidemic (Dullaart, 2017; Boren and Williams, 2016).

Cytokine-targeted therapies, such as TNF or IL-17 antagonists, have transformed treatment of autoimmune diseases, including rheumatoid arthritis and psoriasis. These diseases carry increased cardiovascular risk, but whether the anti-cytokine therapies used to reduce the autoimmune pathology directly impact plaque inflammation and progression is not clear. The recently completed CANTOS trial of an anti-IL-1β blocking antibody (canakinumab) in subjects with a history of myocardial infarction and elevated serum high-sensitivity C-reactive protein concentration (hsCRP) is the first clinical study of the effectiveness of an anti-cytokine therapy given explicitly for cardiovascular disease (Ridker et al., 2017). Treatment of over 10,000 patients with canakinumab was shown to reduce the risk of secondary nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death by 15% compared with compared to placebo. Canakinumab treatment significantly lowered serum hsCRP and IL-6 concentrations but had no effect on lipids, suggesting that the cardiovascular risk reduction was directly related to anti-inflammatory effects. The currently active Cardiovascular Inflammation Reduction Trial (CIRT) will determine if myocardial infarction or stroke in a high-risk population can be decreased by low-dose methotrexate, which is an immunosuppressive regimen targeting cytokine-producing immune cells that has long been used to treat rheumatoid arthritis (Ridker et al., 2012).

A concern for strategies that attack immune activation for a chronic disease process like atherosclerosis is compromise of host defense. Indeed, the risk of fatal infection was significantly increased in the canakinumab-treated group in the CANTOS trial (Ridker et al., 2017). The realization that physiologic mechanisms underlying resolution of inflammation are impaired in the human plaque environment and that pro-resolution therapy may not compromise host defense as much as anti-inflammatory therapy has led to active work in developing therapies based on delivery of pro-resolution mediators into lesions (Fredman et al., 2015; Hasturk et al., 2015; Viola et al., 2016; Petri et al., 2017). Another challenge to treating chronic autoimmune diseases is the accumulation of autoreactive tissue resident memory lymphocytes whose pathogenic phenotypes are stabilized by epigenetic modifications of various gene loci (Phan et al., 2017). There is also evidence that metabolic conditions can induce epigenetic changes in macrophages, leading to a form of innate immune memory ("trained immunity") (Netea et al., 2016; Phan et al., 2017) that may promote lesion progression (Bekkering et al., 2016). In this regard, pre-clinical studies have provided support for the principal that targeting physiologic epigenetic modifiers, for example with inhibitors of Bromodomain and Extra-Terminal motif (BET) proteins, can inhibit inflammatory gene expression and reduce atherosclerosis (Brown et al., 2014; Jahagirdar et al., 2014). Many preclinical studies have validated the concept of treating atherosclerosis with therapeutic vaccinations that tolerize against antigens that drive proatherogenic T cell responses. Recent progress in this area has included identification of apoB100 peptide epitopes that bind mouse or human class II MHC glycoproteins, and the demonstration that vaccination of mice expressing MHC molecules that can bind the peptide reduces lesion formation (Gistera et al., 2017; Kimura et al., 2017). Evidence is provided suggesting the mechanism of protection is induction of Treg cells(Kimura et al., 2017) or antibodies that block LDL activation of macrophages (Gistera et al., 2017). Careful assessment of efficacy and adverse effects of these new therapies, as well as future ones that will undoubtedly be conceived as we learn more about the immune mechanisms that drive

atherosclerosis, represent important ongoing efforts to complement apoB LP-lowering therapy for the prevention and treatment of atherothrombotic vascular disease.

Acknowledgments

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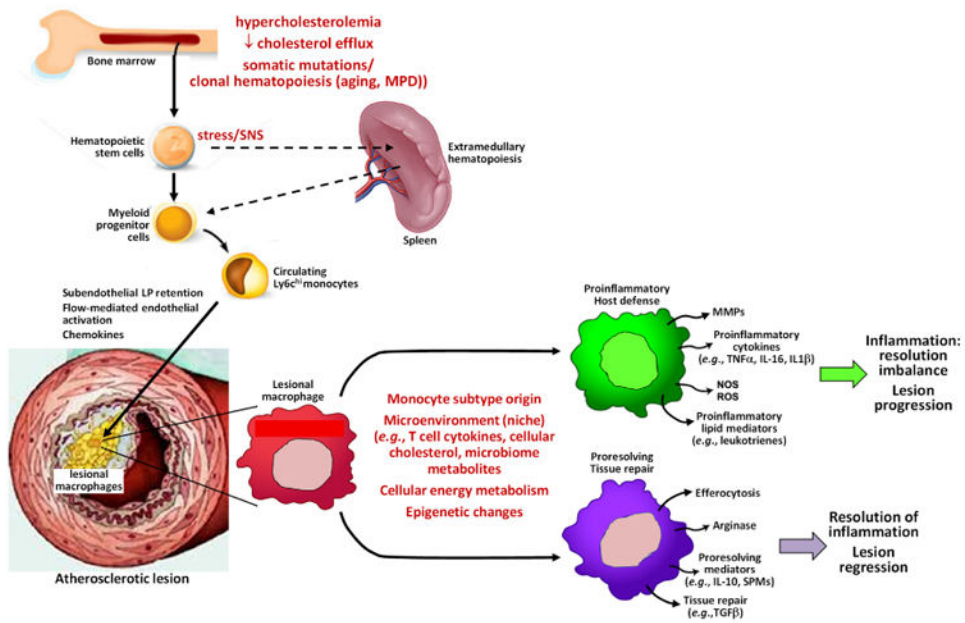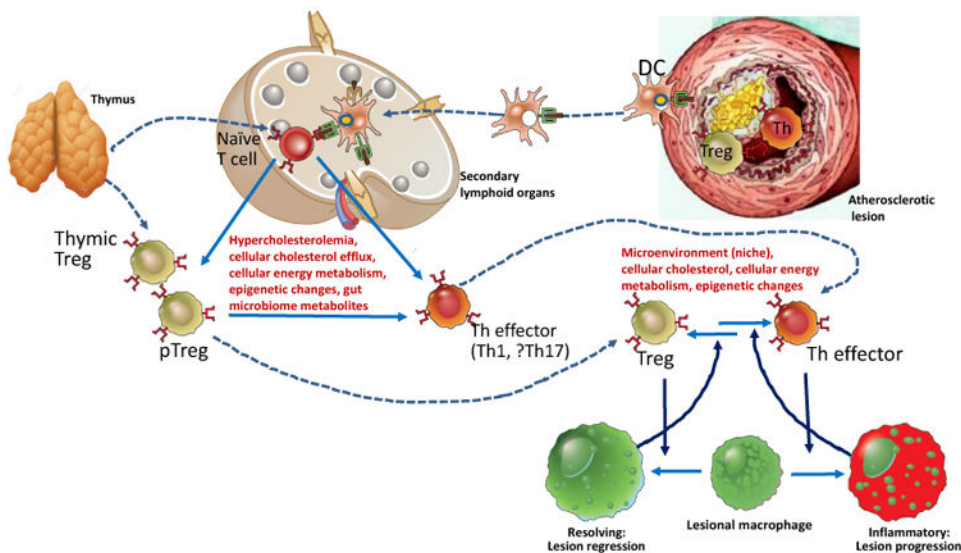
Figure 1. Regulation of Innate Immune Processes Related to Monocyte-Macrophages in Atherosclerosis

Lesional macrophages originate from bone marrow-derived hematopoietic stem cells, which give rise to circulating monocytes. In certain instances, these stem cells first populate the spleen and then undergo extramedullary hematopoiesis. Proliferation and release of hematopoietic stem cells can be exacerbated by elevation of cellular cholesterol and by somatic mutations leading to clonal hematopoiesis, such as those that occur in aging and myeloproliferative disease (MPD). This process can also be stimulated by stress-induced sympathetic nervous system (SNS) activation. The major subpopulation of monocytes that contribute to atherosclerosis progress are Ly6c^{hi} monocytes, which enter lesions in response to subendothelially retained apoB-lipoproteins (LP) and subsequent chemokine release by activated endothelial cells. After differentiation into macrophages, they undergo a variety of phenotypic changes under the influence of the factors listed in the figure. Those macrophages on the inflammatory end of the spectrum secrete proteins and carry out processes that promote atherosclerosis progression, while those on the resolution end of the spectrum promote lesion regression. See text for details.
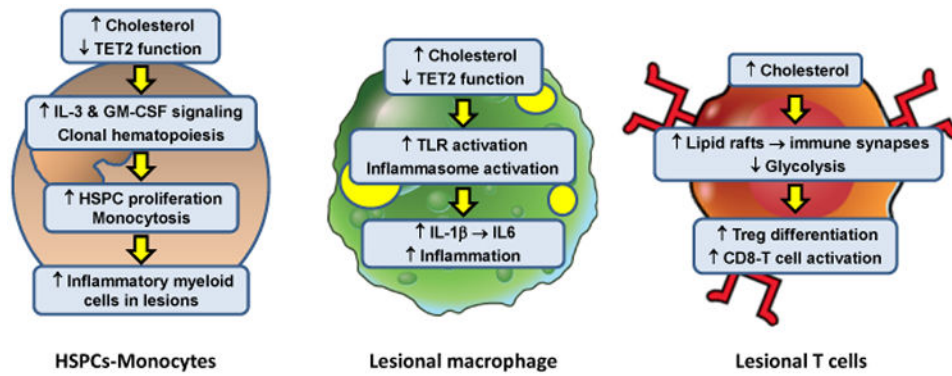
Figure 2. Intracellular Effects of Excess Cholesterol on Myeloid Cells and T Cells That Influence Atherosclerosis

In the setting of hypercholesterolemia or defects in cholesterol efflux, hematopoietic stem and progenitor cells (HSPCs) accumulate excess cholesterol. The consequence is enhanced IL-3 and GM-CSF growth factor signaling, leading to HSPC proliferation and monocytosis. With aging, clonal hematopoiesis can occur owing to loss-of-function mutations in a number of genes, including *TET2*. This process also contributes to monocytosis. Monocytosis is associated with increased accumulation of inflammatory monocyte-derived macrophages in atherosclerotic lesions and higher risk of atherosclerotic vascular disease in humans. These lesional macrophages are also subject to intracellular cholesterol accumulation owing to their internalization of subendothelial apoB LPs. Excess cholesterol in macrophages has multiple effects that enhance lesion inflammation, including toll-like receptor (TLR) and inflammasome activation. The result is increased production of inflammatory chemokines and cytokines, including inflammasome-derived IL-1β and IL-1β-induced IL-6 production. Moreover, changes associated with clonal hematopoiesis, *e.g.*, loss of TET2 function, can also activate the inflammasome in macrophages, further fueling lesional inflammation. T cells do not have the capacity to accumulate large amounts of excess cholesterol, but several studies have shown that perturbations of T cell cholesterol metabolism can affect T cell differentiation and activation. Impaired ABCG1 cholesterol efflux from T cells results in enhanced Treg differentiation which reduces atherosclerotic lesion development and inflammation. In contrast, impaired esterification of cholesterol by deficiency or inhibition of acyl-coenzyme A:cholesterol acyltransferase (ACAT) increases CD8 effector T cell lipid raft formation and thereby enhances immune synapse formation and killing functions of these cells. The net effect of increases in T cell cholesterol on lesion development and inflammation are likely to reflect changes in the Teff:Treg balance and the influence of Teff cells on lesional macrophages.

Figure 3. Regulation and Impact of Adaptive Immune Processes Related to T cells in Atherosclerosis

T lymphocyte responses that affect atherosclerosis include a balance between inflammatory effector T cells, mainly interferon γ-producing T helper (Th) cells, and anti-inflammatory regulatory T cells (Treg). Pro-atherogenic Th effector cells differentiate from thymic-derived naïve T cell precursors in secondary lymphoid organs (SLOs) such as lymph nodes, in responses to antigen presentation of LDL-derived peptides by dendritic cells (DC), some of which may have migrated from the arterial wall. Treg develop in the thymus, and peripheral Treg (pTreg) can also be differentiated from peripheral naive T cells in SLOs. The direction of differentiation of naïve T cells into different Th subsets and Treg in SLOs can be influenced by systemic and local metabolic conditions. Treg and Th cells migrate into developing atherosclerotic lesions and modulate the local inflammatory microenvironment, in large part by influencing macrophage phenotypes. Conversely, resolving or inflammatory macrophage phenotypes can shift the plaque T cell balance toward Treg and Th phenotypes, respectively. Change in Treg-Th cell balance may reflect phenotypic plasticity by permitting re-differentiation between regulatory and inflammatory phenotypes, which is also influenced by systemic and plaque metabolic conditions. See text for details.