Lineage of Bone Marrow–Derived Cells in Atherosclerosis

Hiroshi Iwata, Ichiro Manabe, Ryozo Nagai

Abstract: Atherosclerosis is a chronic inflammatory disease driven by lipids and other atherogenic factors. It is characterized by a dynamic and complex pathological process of bone marrow–derived cells playing divergent roles. Recent studies have begun unraveling the contribution of growing varieties of subsets of immune cells and other bone marrow–derived cells to atherogenesis. For example, bone marrow–derived vascular progenitor cells have been shown to play an important role in the pathogenesis of atherosclerosis. This review provides an overview of the current understanding of contributions of bone marrow–derived cells to atherosclerosis. Particular focus is placed on myeloid cells and vascular progenitor cells. We also summarize the uncertainty surrounding cellular lineage identity and functions. Expansion of our understanding of pathological roles of various subsets of bone marrow–derived cells in atherosclerosis may lead to identification of novel cellular and molecular targets for development of therapeutic strategies. (Circ Res. 2013;112:1634-1647.)

Key Words: atherosclerosis ■ bone marrow–derived cells ■ inflammation ■ progenitors

L eukocyte infiltration into atherosclerotic lesions was first described in the middle 19th century. Rudolf Virchow suggested that inflammation or infiltrated leukocytes directly contribute to the pathogenesis of atherosclerosis, whereas Carl von Rokitansky considered these changes secondary responses.1 After these early observations, numerous studies have worked to unravel the role of immune cells in atherogenesis. Now, it is widely accepted that atherosclerosis is a chronic inflammatory disease.2,3 Chronic inflammatory processes alter the 3-dimensional structure of the vascular wall (vascular modeling), leading to the atherosclerotic plaque formation.4 Various bone marrow (BM)–derived immune cells, including monocytes/macrophages and lymphocytes, are known to be actively involved in the processes. In addition to leukocytes, recent studies have demonstrated that BM cells also may serve as sources of vascular cell lineages, including endothelial progenitor cells (EPCs) and smooth muscle progenitor cells (SMPCs).5,6 Although these vascular progenitors originally had been thought to differentiate into mature and functional endothelial and smooth muscle cells (SMCs) in physiological and pathological settings,5,7 subsequent studies have reported conflicting results.8 Therefore, it is still unclear whether these cells acquire definitive endothelial and SMC identities. Accordingly, their functional contributions to atherogenesis are still undergoing debate. This review summarizes the current understanding of the functional role of BM-derived leukocytes and progenitor cells in the pathological processes of atherosclerosis and neointima formation. Lineages, differentiation, and functions of BM-derived cells in atherosclerosis are summarized in the Figure and Table 1.

In this review, we primarily focus on the functions of cells present within the arterial wall. However, recent studies have shown that atherosclerosis is affected by inflammation and immune responses in distant organs. Atherosclerosis may be considered more appropriately as a manifestation of chronic systemic inflammatory processes that are regulated by organ networks. We advise readers to refer to recent excellent reviews on these matters.9,10

Monocytes and Macrophages

Macrophages have received particular attention in atherogenesis because of early observations that macrophage-derived foam cells centrally contribute to the formation of necrotic core and lesion development in atherosclerosis. Infiltration of circulating monocytes into the subendothelial space via adhesion molecules on activated endothelial cells (ECs) is one of the key processes of plaque formation.11 Previous studies have shown that monocytes that enter the vessel wall become cells with macrophage–like features in Apoe−/− mice, although...
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trolling monocytes.15–18 In humans, 3 monocyte subsets can be identified mainly based on differential expression of Ly-6C, CX3CR1, and CCR2.15 One subset exhibiting Ly-6ChighCX3CR1lowCCR2+ is designated inflammatory monocytes, and the other subset with Ly-6ClowCX-3CR1highCCR2− is designated resident or pa-
trolling monocytes.15–18 In humans, 3 monocyte subsets can be identified mainly based on CD14 and CD16 expression.19 The classical subset exhibiting CD14++CD16− monocyte levels is associated with an increased risk of cardiovascular events in human.20 Hyperlipidemia-induced monocytosis is partly caused by generation of monocytes in BM (medullary he-
matopoiesis). Recently, it also was shown that hematopoietic stem and progenitor cells relocate from the BM to splenic red pulp, in which they expand and differentiate into Ly-6C high.29 This additional pool of monocytes may pro-

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Less is known about the fate of Ly-6C low monocytes within the atheroma. However, these cells were shown to patrol the endothelium for injury and infection by long-range crawl-
ing.32 Ly-6C low monocytes also were shown to be recruited at a late phase to the infarcted myocardium and were suggested to promote wound healing.32 Their functional role in atheroma remains to be elucidated.

Intimal macrophages may internalize oxidized low-den-
sity lipoproteins (LDLs) through scavenger receptors and become foam cells, the main components of the fatty streak. After these early events, the complex interplay between ECs, SMCs, and immune cells, including macrophages and T lymphocytes, further drive lesion progression through

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cytokine signaling networks that mediate the dynamic cellular interactions.

Development of atherosclerotic plaques may involve 2 scenarios that are not necessarily mutually exclusive. When the balance between proinflammatory and anti-inflammatory populations of accumulating BM-derived cells in the cellular processes is tipped toward anti-inflammatory reactions that promote healing, the plaque may become stabilized by a thick and stable fibrous cap. Alternatively, active inflammation is thought to result in instability of the plaque by promoting thinning of the fibrous cap that is then prone to plaque rupture and sudden lumen occlusion by thrombus. Unstable plaques typically exhibit active inflammatory processes that are characterized by constant infiltration of inflammatory cells and production of inflammatory cytokines. The prolonged accumulation of apoptotic cells, cell debris, and cholesterol crystals promotes progression of the necrotic core, in which apoptotic macrophages are the major constituent. Moreover, macrophages produce inflammatory cytokines and matrix metalloproteinases (MMPs), which promote inflammation and plaque vulnerability. As such, macrophages are the major active player in determining the evolution of atherosclerotic plaque.

An important function of lesion macrophages is to remove excess lipid, and the lipid-laden macrophages that appear within the plaque are referred to as foam cells. Macrophages may take-up natural and modified lipoproteins, such as acetylated LDL, oxidized LDL, and glycolaldehyde-LDL, as well as LDL enzymatically altered by trypsin, cholesterol esterase, and neuraminidase. These modified forms of LDL have been shown to promote foam cell formation and atherogenesis by stimulating secretion of various cytokines. This leads to the further recruitment of inflammatory cells, resulting in formation of necrotic core.

Excess cholesterol ester accumulation in macrophages of the vascular lesions occurs as a result of an imbalance between delivery and removal. Macrophages are incapable of limiting the uptake of lipids and, therefore, largely depend on cholesterol efflux pathways to maintain cellular lipid homeostasis. In early atherogenesis, macrophage apoptosis is associated with reduced atherosclerosis progression attributable to effective efferocytosis by neighboring phagocytes, which, in turn, reduces proinflammatory mediators present in the lesion. However, as atherosclerosis progresses, efferocytosis becomes impaired. Potential contributing mechanisms for impaired efferocytosis include oxidative stress–induced death of efferocytes attributable to the overabundance of apoptotic cells withins the plaque and dysfunction of the efferocytosis receptor MerTK. A failure of effective efferocytosis leads to secondary necrosis, in which macrophages die and release their cellular contents, including debris, oxidized lipids, and proinflammatory mediators. Secondary necrosis amplifies the inflammatory response and accelerates the development of a necrotic core in the plaque.

In response to various local environmental cues, macrophages are now known to assume divergent phenotypes. In vitro studies have shown that Th1 cytokines alone, or in concert with microbial products, elicit classical M1 activation of macrophages, whereas Th2 cytokines (interleukin [IL]-4 and IL-13) elicit an alternative form of activation designated M2. A failure of effective efferocytosis leads to secondary necrosis, in which macrophages die and release their cellular contents, including debris, oxidized lipids, and proinflammatory mediators. Secondary necrosis amplifies the inflammatory response and accelerates the development of a necrotic core in the plaque.
Phenotypic switching in macrophages from M2 to M1 also was found in atherosclerosis on induction of disease regression.99

Although the M1 and M2 classifications highlight the 2 extremes of macrophage activation, it is becoming increasingly clear that macrophages can exhibit highly divergent phenotypes and functions in vivo. In particular, M2 may include various forms of macrophage activation.90 Other types of macrophage polarization have been described. Mox phenotype develops in response to atherosclerotic phospholipids via expression of the redox-regulated transcription factor Nrf2 and has a lower phagocytic and chemotactic capacity than the conventional M1 and M2 macrophages.91 Moreover, it also has been proposed that Mhem, which is activated by intraplaque hemorrhage, is often associated with atherothrombotic complications.51,52 M4 is a recently proposed subtype of atherogenic macrophages induced by CXCL4.93 As such, the classification of macrophages in atherosclerosis does not seem to be as simple as previously proposed. Furthermore, there is a significant gap in our understanding between macrophage polarization in vitro and functional roles in vivo. Therefore, future studies will need to address the functional contributions of differentially activated macrophages to the physiology and pathology of the blood vessel.94

Vascular Progenitor Cells

After the discovery of EPCs in circulation in mice,5 various circulating progenitor cell populations that can acquire EC-like or SMC-like phenotypes have been identified in mouse and human.75,95 These cells have been shown to exhibit similarities in surface markers and functions with myeloid cells.5,8,56,57 However, their origin, identity, and function are still poorly understood, and a unified definition of EPCs and SMPCs has not yet been established. It is, in fact, still undergoing debate which populations are bona fide progenitor cells and whether these cells are even capable of differentiating into definitive vascular lineages in vivo. Moreover, physiological and pathological functions of EPCs and SMPCs are not well-understood, partly because they are assumed, a priori, to acquire EC or SMC functions. Irrespective of whether they are bona fide progenitor cells, numerous studies have identified BM-derived cells that express some of the endothelial or SM genes within vascular lesions. Therefore, it is important to assess whether and how they contribute to vascular disease.

Endothelial Progenitor Cells

Asahara et al96 published a landmark article in 1997 showing that BM-derived CD34+VEGFR-2+ peripheral mononuclear cells (MNCs), isolated from human blood and grown in culture are able to differentiate into cells expressing endothelial markers, including CD31, E-selectin, and endothelial nitric oxide synthase. Subsequently, CD34+ cell-enriched MNCs transplanted into mouse and rabbit models of hindlimb ischemia were shown to promote neovascularization and incorporate in the capillary walls, suggesting they contribute to blood vessel formation in adult.98 Based on these findings, the cells were designated as EPCs.

Although other sources of EPCs, such as resident progenitor cells in blood vessel wall,99 adipose tissue,100 and spleen,101 were proposed, BM has been considered as the main reservoir of these specialized cells. Various methods to identify EPCs have been developed: methods of isolation, cell surface markers, and functions in angiogenesis and in atherosclerosis of EPCs are summarized in Table 2. Currently, the most commonly used method to identify and distinguish EPCs from mature ECs is flow cytometric analysis of expression of cell surface antigens, such as CD34, kinase insert domain–containing receptor (KDR), VE-cadherin, von Willebrand factor, E-selectin, and CD133.62 Different combinations of cell surface markers may identify different cell populations.

The second commonly used identification method for EPCs involves culturing circulating MNCs and monitoring phenotypic changes over time. One subtype of EPCs arises 3 to 5 days after seeding of MNCs (early EPCs63,64 and colony forming unit ECs). Another type of EPCs appear after 2 to 3 weeks of culture (late EPCs or outgrowth ECs [OECs]).54,66 Two subtypes seem to be substantially distinct in multiple respects. First, late EPCs or OECs have more similarities to mature ECs in not only expression of cell surface antigens, such as von Willebrand factor, KDR, and KDR, but also functional aspects, including high levels of endothelial nitric oxide synthase synthase production and effective participation in tube-forming assays in vitro as compared with early EPCs. Moreover, late EPCs or OECs are potent sources of several growth factors, cytokines, and chemokines, such as vascular endothelial growth factor, IL-8, hepatocyte growth factor, granulocyte colony–stimulating factor, and granulocyte macrophage colony–stimulating factor.67 Finally, early EPCs are mostly derived from the CD14+ population of MNCs, whereas OECs derive mostly from the CD14+ population.68

Functionally, early EPCs are suggested to participate in angiogenesis in a paracrine fashion like endothelial-like macrophages, rather than highly differentiated ECs.99 Late EPCs or OECs are suggested to directly participate in angiogenesis like cells composing blood vessels.70,71 Although these time-dependent culture methods have demonstrated that ex vivo culture of circulating MNCs produce cell populations that resemble ECs, these results may not necessarily indicate that the same subsets of MNCs differentiate into ECs in vivo. Long-term culture has been shown to greatly modulate epigenome72 and may induce aberrant gene expression that would not occur in vivo settings. Purhon et al73 demonstrated that no BM-derived cells incorporated into vascular ECs in tumor angiogenesis as well as matrigel assay. As such, whether BM-derived cells acquire a definitive EC lineage is still undergoing debate. In fact, many phenotypic characteristics and behaviors that are attributed to ECs are shared by myeloid cells.74 Moreover, monocytes/macrophages support and promote angiogenesis during development and in pathologies.75 Further lineage tracing experiments are needed to clarify the cell fates of circulating MNCs in vivo.

Circulating EPCs are generally considered protective in the pathogenesis of atherosclerosis as well as neointima hyperplasia after vascular injury. Observational clinical studies of patients with coronary artery disease showed a significantly lower incidence of adverse clinical events in
individuals with higher numbers of circulating CD34+KDR+ cells.76,77 HMG-CoA reductase inhibitors (statins) and physical exercise, which are known to improve EC function, were shown to increase the number of circulating EPCs.78–80 A beneficial effect of EPCs also was shown in animal studies. For instance, in a rabbit balloon injury model, the transfer of ex vivo cultured EPCs significantly suppressed neointima hyperplasia.81 Although these studies suggest beneficial roles of EPCs, some evidence indicated unfavorable effect of EPCs, such as promoting plaque progression and instability.82,83 We also previously reported that the presence of a higher population of CD34+ cells in coronary thrombi of patients with myocardial infarction had higher incidence of restenosis after coronary intervention.84 These findings suggested an increased incidence of restenosis after successful coronary intervention in these patients, indicating that CD34+ cells in thrombi might promote neointimal hyperplasia after angioplasty. A potential reason for these discrepant roles of EPCs may arise from the fact that each study used differently identified cell populations than EPCs. In fact, the unequivocal definition and isolation method of circulating EPCs have not been established.85 Most cell surface antigens that are currently used to identify EPCs can be expressed by other cell types, including hematopoietic stem cells.86 Moreover, several studies suggest that myeloid progenitors have the capacity to differentiate into vascular ECs,87 whereas others propose that BM-derived cells are recruited to a perivascular position and act as paracrine cells that promote angiogenesis but do not differentiate into definitive ECs.88,89 As described, early EPCs and late EPCs seem to be functionally different. Accordingly, the impact of EPCs on physiology and pathology of the blood vessel remains unclear. Because current identification methods may isolate cells that do not have potential for differentiating into definitive ECs in vivo, all EPCs cannot be uniformly considered EPCs. At least some of them may have different functions than definitive ECs. As such, it may be important to carefully reevaluate the functional roles of each EPC subset in vascular disease and physiology.

### SMPCs

Circulating SMPCs were identified as circulating BM-derived cells that are recruited to blood vessels and acquire phenotypes expressing SMC marker genes, particularly SMα-actin.70–72 These SMPC-derived cells are often called SM-like cells (SMLCs). In humans, donor-derived SMLCs were detected throughout atherosclerotic vessels in patients who received sex-mismatched BM transplantation, suggesting the involvement of circulating SMPCs in intimal proliferation.73 To date, no specific cell surface or intracellular markers that differentiate SMPCs and SMLCs from SMCs have been established. Therefore, these cells are largely identified as circulating or resident cells within the BM, vascular wall, or perivascular area that acquires phenotypes with some similarities to SMCs.

There are a number of SMC differentiation marker genes with different specificity, including SMα-actin, SM22α, SM-calponin, smoothelin, and SM-myosin heavy chain (SM-MHC).74 One of the difficulties in identification of SMC lineage is the plasticity in phenotypes of SMCs. SMCs can greatly alter their phenotypes during development and vascular disease. Another problem is that all SMC differentiation markers can be expressed by non-SMCs. Among SMC markers, SM-MHC is the most stringent marker for SMC lineage.75 The most commonly used SMC marker, SMα-actin, can be expressed by various non-SMCs, including ECs, fibroblasts, and macrophages. Therefore, using SMα-actin as a sole marker is insufficient to identify SMC lineage. Additionally, it also was shown that phenotypically modulated SMCs can express macrophage markers.76 As such, it is not trivial to unequivocally identify SMC lineages in vascular lesions.

Using multiple SMC differentiation markers, including SM-MHC, we carefully analyzed phenotypes of BM-derived SMLCs.8 We found that BM-derived SMα-actin+ cells in vascular lesions in mice do not express the definitive SMC lineage marker, SM-MHC, even under conditions in which resident SMCs expressed SM-MHC. Because SM-MHC expression in resident SMCs is downregulated in the neointima, we analyzed lesions 12 months after vascular injury. Even at this late stage during which resident SMCs

### Table 2. Summary of Isolation Methods of EPCs and Their Functions in Angiogenesis and Atherosclerosis

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>Adherent/Nonadherent in Initial Culture</th>
<th>Culture Period, d</th>
<th>Selection of Subpopulation</th>
<th>Endothelial Markers (CD31, CD144, KDR, vWF)</th>
<th>Myeloid Markers (CD14, CD45)</th>
<th>Angiogenesis</th>
<th>Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early EPC</td>
<td>Adherent</td>
<td>&lt;5</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>Promote angiogenesis in paracrine manner5</td>
<td>Generally considered as atheroprotective56 but several reports indicate proatherogenic properties of EPCs57,58</td>
</tr>
<tr>
<td>Late EPC (outgrowth EPC)</td>
<td>Adherent</td>
<td>&gt;14</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>Differentiate into endothelium45</td>
<td></td>
</tr>
<tr>
<td>CFU-EC (CFU-Hill)</td>
<td>Nonadherent</td>
<td>&lt;10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Promote angiogenesis in paracrine manner45</td>
<td></td>
</tr>
<tr>
<td>ECFC</td>
<td>Adherent</td>
<td>&gt;14</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>Differentiate into endothelium, high proliferation capacity57</td>
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</tbody>
</table>

CFU-EC indicates colony-forming unit-endothelial cell; ECFC, endothelial colony-forming cells; EPC, endothelial progenitor cells; KDR, kinase insert domain-containing receptor; and vWF, von Willebrand factor.
fully recover SM-MHC expression, the BM-derived cells did not express SM-MHC. Instead, the BM-derived SMα-actin+ cells express markers of monocytes/macrophages. Moreover, we found that adoptively transferred CD11b-Ly-6C− BM monocytes expressed SMα-actin in the injured artery. These results strongly suggest that a subset of myeloid lineage cells can acquire SMC-like phenotype characterized by SMα-actin expression. However, they do not acquire the definitive phenotype of SMC lineage in the models we used, including wire injury, heart transplantation, and Apoe−/−mice. Expression of inflammatory genes and MMPs in these SMα-actin–positive cells suggests that they contribute to the remodeling processes.

Our results indicate that myeloid cells from BM can acquire phenotypes partly resembling SMCs. Interestingly, it also was reported that SMCs can express genes that are expressed in macrophages. Cholesterol or oxidized LDL loading of SMCs resulted in a marked suppression in expression of SMC markers in cultured SMCs. This SMC dedifferentiation was followed by activated expression of multiple macrophage markers, including CD68, Mac-2, and ABCA1, and macrophage-like phagocytic activity. These results suggest that a part of macrophage marker–positive cells within vascular lesions may be of the SMC origin. These findings point to the need to reevaluate the cell lineages of SMCs and macrophages in vascular lesions. We need to clarify which cells within atherosclerotic lesions are of BM or SMC origin, and what roles these cells might play in lesion development, progression, and plaque instability or rupture.

Functions of SMPCs in the process of atherogenesis are not well-understood. Several reports suggest that SMPCs promote inflammation and plaque instability by producing cytokines and MMPs. It also was suggested that SMPCs are involved in pathological angiogenesis in the atherogenic plaque. In contrast, athero-protective effects of SMPCs have been rarely reported, although in theory they might contribute to the formation of fibrous cap. Clearly, further studies are needed to better-understand phenotypic and functional features of SMPCs in the vascular disease.

Circulating Fibrocytes
Fibrocytes are BM-derived mesenchymal progenitors that coexpress markers of hematopoietic stem cells, the monocyte-lineage, and fibroblasts. They produce extracellular matrix (ECM) components as well as ECM-modifying enzymes and can further differentiate into myofibroblast-like cells. Fibrocytes are suggested to promote local inflammation via antigen presentation, cytokine and chemokine secretion, and production of MMPs. A gene expression profile study of human fibrocytes identified signature genes, including TLR4, IL-1β, CCL2, CCL3, CCL7, CCL22, and C5aR, suggesting that fibrocytes participate in an inflammatory response.

In vitro studies showed that fibrocytes are derived from CD14+ BM-derived monocytes in humans and from the Gr1+CD11b−CD11c+ monocyte population in mice. Therefore, it was proposed that circulating fibrocytes are a transitional cell population between monocytes and fibroblasts. Interestingly, they also were shown to have potential to dedifferentiate back to monocytes or dendritic cells (DCs). Moreover, human fibrocytes were shown to be capable of differentiating into cells with characteristics of adipocytes, chondrocytes, myofibroblasts, and osteoblasts. As such, fibrocytes may differentiate along multiple mesenchymal lineages, but it is less clear which differentiation paths are applicable in vivo. More importantly, their lineage identity, particularly relative to monocytes, is not clear. Clearly, further work is needed to better-define the lineage of fibrocytes.

In human peripheral blood, 0.1% to 0.5% of nucleated cells were identified as circulating fibrocytes that express type 1 and 2 collagens, vimentin, and, occasionally, SMα-actin. Because no single marker that unequivocally identifies fibrocytes has been established, combinations of expression of collagen and other surface markers, including CD34, CD45, and CD68, have been used. More recently, a combination of CD45RO, 25F9, and S100A8/A9 or CD49 also was used as a marker. However, it also was reported that there are considerable overlaps in the gene expression profiles among human monocytes, macrophages, fibrocytes, and fibroblasts.

In atherosclerotic lesions, fibrocytes expressing procollagen I and CD34 have been identified in the fibrous cap of human atherosclerotic lesions. Subendothelial SMα-actin–positive myofibroblasts expressing the monocyte marker CD68 have been found in lipid-rich areas of the atherosclerotic intima in human aorta. One of the subsets of monocytes that are preferentially recruited to sites of atherosclerosis is the inflammatory subset of CD14+CD16−MNCs expressing CCR2, suggesting that the CD14+CD16−CCR2+ subpopulation may be involved in atherogenesis and may contain precursors of fibrocytes that contribute to the development of atherosclerosis. Marker expression profiles suggest that there may be considerable overlaps between fibrocytes and SMPCs/SMLCs; therefore, it may be important to clarify the relationship in lineages and functions between fibrocytes and SMPCs to better-understand the common physiological and pathological roles of BM-derived SMC-like and fibroblast-like cells in the response of the body to insults.

The expressions of collagen and other ECM proteins by fibrocytes suggest that accumulation of these cells in the fibrous cap might stabilize the plaque. However, fibrocytes also are known to express inflammatory cytokines and MMPs. As such, future studies need to clarify functional roles of fibrocytes in the course of atherogenesis and plaque destabilization. Identification of BM-derived circulating EPCs, SMPCs, and fibrocytes has suggested more divergent roles of BM-derived cells than previously considered. However, it is also clear that our understanding of their lineages and functions is still limited. In particular, it would be important to further analyze their functional contribution to lesion evolution.

DCs
DCs are specialized antigen-presenting cells. Classical DCs differentiate from common DC precursors in BM and are present in both lymphoid and nonlymphoid tissues at steady-state. Classical DCs in nonlymphoid tissues are highly migratory and can move from peripheral tissues to the draining lymph nodes where they interact with T cells. Plasmacytoid DCs also arise from common DC precursors.
and are characterized by rapid production of type I interferons (IFNs) in response to viral infection. They are present in all peripheral organs, including the aorta. Although monocytes are not thought to differentiate into classical DCs, subpopulations of DCs have been suggested to arise from monocytes. However, the relationship between DCs and monocytes and macrophages, particularly under conditions of inflammation, is still undergoing debate. Surface expression of CD11c is often used to identify DCs. However, CD11c is also expressed in a variety of tissue macrophages. Likewise, the markers that have been used to distinguish macrophages from DCs, such as F4/80, CD11c, CD11b, and MHC II, are not specific. Moreover, macrophages can present antigens.

In mouse and human aortae, classical DCs are present in steady-state. In mouse, 2 subpopulations of DCs were identified: CD103+CD11b−F4/80− classical DCs, whose presence depends on Flt3 and Flt3 ligand signaling, and CD103−CD11b+F4/80+DC-SIGN+ monocyte–derived DCs. Both populations were increased in atherosclerosis in the aortae of Ldlr−/− mice. In early Ldlr−/− atherosclerotic lesions, the majority of intimal proliferating cells were positive for DC markers, including CD11c, MHC class II, and 33D1, but were low in CD11b−. These results demonstrate that classical DCs are present in atherosclerotic lesions.

Whether a DC will activate or inhibit T cells depends on its pattern of cytokine release and expression of cell surface costimulatory molecules. DCs therefore are a crucial link between innate and adaptive immune responses. The functional role of DCs in atherosclerosis is not fully understood but is still undergoing debate. Surface expression of CD11c is often used to identify DCs. However, CD11c is also expressed in a variety of tissue macrophages. Likewise, the markers that have been used to distinguish macrophages from DCs, such as F4/80, CD11c, CD11b, and MHC II, are not specific. Moreover, macrophages can present antigens.

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Mast Cells

Mast cells are widely distributed throughout the vascularized tissues and are well-positioned to be one of the first cell types to interact with environmental antigens and allergens. These cells also are localized in the vessel walls, particularly within the adventitia. Clinical studies demonstrated the abundant presence of mast cells in the intima and adventitia of atherosclerotic plaques in the diseased coronary arteries. In human coronary arteries, colocalization of activated mast cells with microvessels in the plaque suggested their role in intraplaque hemorrhage and plaque destabilization. Furthermore, oxidized LDLs induce mast cell degranulation and release of cathepsins, heparin, histamine, and a variety of inflammatory cytokines, resulting in exacerbation of atherosclerosis. Mast cell activation in ApoE−/− mice causes plaque instability via release of proteolytic enzymes, such as tryptase, and metalloproteinases. Collectively, current evidence indicates that mast cells are generally proatherogenic and also promote plaque instability. In addition to their function as effector cells, growing evidence indicates that mast cells can positively or negatively modulate immune responses. Mast cells have been shown to produce proinflammatory cytokines, including IL-6 and IFN-γ, and promote atherosclerosis. In contrast, their negative regulatory role has not been addressed in atherosclerosis.
Lymphocytes

Lymphocytes also have been shown to play important roles in the complex inflammatory processes in the vascular wall.\textsuperscript{145} A simplified view is that proinflammatory Th1 and cytotoxic T cells accelerate atherogenesis, whereas regulatory T and Th2 cells are protective.\textsuperscript{146} However, as shown in other fields, each T-cell subset may have different roles in different phases of atherosclerosis.

Helper T-Cell Subsets

CD4\(^+\) Th cells have been shown to play key roles in regulating the atherosclerotic processes. In Apoe\(^{-/-}\) mouse on a severe combined immunodeficiency background, supplementation of CD4\(^+\) T cells aggravated atherosclerosis.\textsuperscript{147} Conversely, absence of CD4\(^+\) cells reduced plaque burden in Apoe\(^{-/-}\) mice.\textsuperscript{148} In contrast, Elhage et al\textsuperscript{149} demonstrated that absence of CD4\(^+\) population significantly aggravated atherogenic change in descending thoracic and abdominal aorta in Apoe\(^{-/-}\) mice, whereas it did not affect lesions in the aortic root. These results suggested that the different populations of CD4\(^+\) T cells may have different roles in atherosclerosis. A number of studies have shown that Th1 cells play a major role in atherosclerotic immune responses.\textsuperscript{150,151} Th1 cells produce proinflammatory cytokines, such as IFN\(\gamma\) and tumor necrosis factor-\(\alpha\), which have been shown to promote atherogenesis.\textsuperscript{152,153} Deficiency of the transcription factor T-box expressed in T cells, which is important for Th1 and Th17 differentiation, reduced atherosclerosis in Ldlr\(^{-/-}\) mice.\textsuperscript{150} These results indicate that Th1 cells are proinflammatory and proatherogenic.

Th2 cells represent a lineage of CD4\(^+\) T cells that primarily interact with B cells. The role of Th2 cells in atherosclerosis seems to be variable, with studies indicating that these cells promote,\textsuperscript{154} protect against,\textsuperscript{155} or have no effect\textsuperscript{154} on atherosclerosis depending on experimental models. In addition, IL-4, the signature cytokine of Th2, has been shown to be atheroprotective and is not frequently observed in human plaques. As such, the role of Th2 immune responses in atherosclerosis is still undergoing debate.\textsuperscript{156}

Another subpopulation in CD4\(^+\) T cells that was recently suggested to be involved in atherogenesis is IL-17–producing T cells (Th17 cells). However, their role in the atherosclerosis process remains controversial.\textsuperscript{157,158} Although several studies showed their proatherosclerotic role in mice,\textsuperscript{156} Th17 cells were not observed in human plaques.\textsuperscript{159}

Tregs

Tregs are a heterogeneous group of immune-inhibitory cells that play a role in suppressing pathogenic immune responses. In atherosclerotic plaques, Tregs are found in very low numbers compared with other chronically inflamed tissues, suggesting that local immune tolerance is impaired in the plaques.\textsuperscript{160} The atheroprotective role of Tregs has been demonstrated using immunologic deletion of Treg cells in Ldlr\(^{-/-}\) and Apoe\(^{-/-}\) mice.\textsuperscript{161,162} Anti-inflammatory cytokines, IL-10, and transforming growth factor-\(\beta\) are considered to be the major protective mediators produced by Tregs.\textsuperscript{163,164} However, these cytokines are known to be produced by other cell types. A recent study suggested a novel antiatherosclerotic function of Tregs.\textsuperscript{165} In this article, Klingenberg et al\textsuperscript{166} showed that deletion of Foxp3\(^+\) Tregs increased atherosclerotic lesions without enhancing vascular inflammation in Ldlr\(^{-/-}\) mice. The mice also exhibited increased plasma cholesterol levels attributable to reduced clearance of very-low-density lipoprotein and chylomicron remnants, suggesting that Foxp3\(^+\) Tregs modulate lipoprotein metabolism. Results of studies on clinical significance of Tregs in atherosclerosis in human are conflicting.\textsuperscript{166,167} More studies are needed to elucidate the role of Tregs in atherosclerosis in human.

CD8\(^+\) T Cells

CD8\(^+\) T cells are present within atherosclerotic plaques.\textsuperscript{168,169} However, the impact of CD8\(^+\) T cells on atherosclerosis development is less established than that of Th1 cells.\textsuperscript{149,170} For instance, CD8\(^+\) T-cell deficiency did not affect plaque burden in Apoe\(^{-/-}\) mice.\textsuperscript{149} CD8\(^+\) T-cell populations in atherosclerosis include cytotoxic lymphocytes and possibly Tregs (CD8\(^+\) Tregs). A potential pathological role of cytotoxic CD8\(^+\) T cells is to aggravate local inflammation by attacking vascular cells, such as SMCs, promoting both lesion progression\textsuperscript{171} and plaque instability.\textsuperscript{172} On the contrary, the CD8\(^+\) suppressor population (CD8\(^+\) Treg) has been shown to have a protective role in atherosclerosis.\textsuperscript{173} Therefore, the accumulating evidence suggests that, in addition to CD4\(^+\) T cells, CD8\(^+\) T cells also play an important role in modulating arterial injury responses.

Natural Killer T Cells

Natural killer T (NKT) cells are a distinct subset of T lymphocytes coexpressing cell surface molecules of natural killer (NK) cells (eg, NK1.1 and Ly49) and T-cell receptors. NKT cells are unique in their ability to respond to glycolipid antigens presented by the major histocompatibility complex class I–like CD1d molecule.\textsuperscript{174} After activation, NKT cells are able to rapidly and robustly secrete large amounts of both proinflammatory and anti-inflammatory cytokines, thereby playing an important regulatory role in a number of pathological states.\textsuperscript{175} Because the atherosclerotic lesion is characterized by the retention and modification of lipids in the vascular wall, their ability to recognize lipid antigens suggests NKT cells may be involved in the vascular inflammatory response. There are 2 classes of NKT cells. The majority of NKT cells express a semi-invariant T-cell receptor and are designated invariant NKT cells. Smaller numbers of NKT cells express more diverse T-cell receptors and are referred to as variant NKT cells. In humans, NKT cells were found at the shoulder regions of carotid artery plaques as well as in atherosclerotic tissue in abdominal aortic aneurysms.\textsuperscript{176} Experimental mouse models demonstrated a proatherogenic role of NKT cells. Invariant NKT cell–deficiency attenuated atherosclerosis in Apoe\(^{-/-}\) mice.\textsuperscript{177,178} In addition, the exogenous administration of the glycolipid, \(\alpha\)-galactosylceramide, that activates NKT cells resulted in a marked increase in plaque burden in Apoe\(^{-/-}\) and Ldlr\(^{-/-}\) mice.\textsuperscript{179}

B Cells

B cells are present in atherosclerotic lesions, although they are less frequent than T cells. Although B cells seem to initially
accumulate in the intima of early atherosclerotic lesions, B cells are more prevalent in the adventitia than in the intima in advanced atherosclerotic lesions.180 B cells with other immune cells may form tertiary lymphoid organs, which promote lymphocyte recruitment and facilitate the immune response against stimulating antigens locally in adventitia in humans and in mice.181,182 Interestingly, adventitial inflammation has been shown to associate with intimal inflammation and was suggested to affect intimal inflammation.183,184 Adventitial B cells and tertiary lymphoid organs therefore may affect inflammatory responses in not only adventitia but also intima.

B cells have been suggested to be either atheroprotective or proatherogenic. The atheroprotective effect of B cells was originally suggested by a study demonstrating significant attenuation of atherosclerosis in Apoe<sup>−/−</sup> and Ldlr<sup>−/−</sup> mice by adoptive transfer of B cells.185,186 Mechanistically, autoantibodies produced by B cells, antioxidants LDL IgM,187 for example, may inhibit oxidized LDL uptake by macrophages and prevent foam cell formation.188 On the contrary, immunologic depletion of B cells was shown to decrease atherosclerotic development in mice.189,190 This apparent discrepancy in B-cell function in atherosclerosis may be explained partly by the unique roles of specific B-cell subsets in atherogenesis, such as B1a B cells (atheroprotective),191 B2 B cells (proatherogenic),190 and regulatory B cell (atheroprotective).192–194 It is also possible that specific antibodies produced by B cells may either promote or suppress atherogenesis.189,195 Regulatory B cells are a B-cell subset that produces IL-10 and have been suggested to regulate inflammation. Their contribution to atherosclerosis remains to be addressed.

**Innate Lymphoid Cells**

Innate lymphoid cells (ILCs) are newly identified members of the lymphoid lineage and have emerged as important effectors of innate immunity and tissue remodeling. ILCs are characterized by 3 main features: the lack of rearrangement of antigen receptors; the lack of myeloid and DC markers; and lymphoid morphotype.196 They are subdivided into 3 subsets, group 1 ILCs (ILC1 and NK cells), group 2 ILCs (ILC2 cells), and group 3 ILCs (ILC3 and lymphoid tissue–inducer cells), based on their ability to produce type 1 (eg, IFN-γ), type 2 (IL-5 and IL-13), and Th17 cell–associated cytokines (IL-17 and IL-22), respectively. NK cells are the prototypical member of group 1 ILCs. Group 2 ILCs have been shown to be important for host defense against nematodes, and also have been shown to be involved in the repair of damaged respiratory tissue.197 Group 3 ILCs include lymphoid tissue–inducer cells that are essential for lymph node development and other ILC3 cells. Except for NK cells, functions of newly identified ILCs in atherosclerosis are unknown.

**NK Cells**

NK cells, a key cellular component of innate immunity, participate in the defense against viral function, as well as pathogenesis of autoimmune diseases by their cytolytic activity and cytokine production.198 The role of NK cells in atherosclerosis is not well-understood; however, their production of proinflammatory cytokines, including IFN-γ, tumor necrosis factor-α, and granulocyte macrophage colony-stimulating factor, suggests proatherosclerotic functions.199–200 Accordingly, inhibition of NK cells attenuated atherosclerosis in Ldlr<sup>−/−</sup> mice,201 and the shoulder region of human plaques was shown to contain modest numbers of CD56<sup>+</sup> NK cells.202 However, a clinical study found a significant reduction of circulating NK cell number in patients with unstable or stable coronary artery disease in comparison with healthy controls, suggesting a potential atheroprotective role of NK cells.203

**Conclusions**

Many studies in animals and in humans have demonstrated that various lineages of BM-derived cells in the circulation infiltrate vascular lesions. In addition to vascular progenitor cells, new subsets of immune cells are emerging as important effectors and regulators of immune responses. Moreover, recent studies have unraveled dynamic plasticity in their phenotypes and functions. Accordingly, we can expect rapid expansion of knowledge of the physiological and pathological roles of these new players. However, the limited surface markers and other lineage markers available often hinder unequivocal identification of cellular identities. This is particularly the case for myeloid lineage cells, which may include EPCs and SMPCs as well. Further assessment of lineages is important not only for clarifying cell identity but also for better understanding of complex interactions between different cell types and the microenvironment. These factors may further affect the recruitment of other cells to the atherosclerotic plaque and play a role in altering their phenotypes. Another important issue is the correspondence between mouse and human cells. In particular, human counterparts of newly identified mouse immune cell subsets may not have been well-characterized.

More importantly, human atherosclerosis develops differently from mouse atherosclerotic models in many aspects. For instance, human atherosclerosis begins as the deposition of extracellular lipid within the deep layer of intima of diffuse intimal thickening that consists of SMCs, elastin, and proteoglycans.204 Macrophages infiltrate into the intima, and a part of them becomes foam cells at the middle layer of the intima. In contrast, the proliferation of SMCs and accumulation of extracellular matrices and lipid droplets occur after the accumulation of foamy macrophages in mouse atherosclerosis, and foam cell macrophages often occupy the whole thickness of the intima as a predominant component of the lesion. Accordingly, interplays between immune cells, vascular cells, and microenvironment are likely to be different in humans compared with mice. In addition, atherosclerotic lesions in the mouse models seldom cause plaque disruption with thrombosis. Needless to say, mechanisms identified in mouse models need to be evaluated in human atherosclerosis. In this regard, rapid advances in experimental technologies (eg, proteomics, metabolomics, and genomics) and diagnostic modalities may facilitate development of biomarkers that bridge animal experiments and human diseases.21 Future studies of the molecular control of the networks of BM-derived cells and vascular cells in atherosclerosis also may lead to identification of novel therapeutic targets.

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Disclosures

None.

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