Atherosclerosis contributes to major mortality and morbidity of cardiovascular diseases in Western countries. Lipid-laden macrophage accumulation in the subendothelial area of the arterial wall is a hallmark of atherosclerosis. These lipid-laden cells promote inflammatory responses in the arterial wall and lead to multiple fatal pathological consequences, such as hemorrhage, rupture, and calcification.\(^1\)\(^-\)\(^3\) Mechanisms of atherosclerosis relating to disruption of lipoprotein metabolism and inflammation have been the major focuses of atherosclerosis research.\(^4\)\(^-\)\(^5\) Animal models are still the major tools to determine mechanisms and to discover potential targets for therapeutic purpose. Although hypercholesterolemic mouse models such as low-density lipoprotein (LDL) receptor–deficient and apolipoprotein (apo) E–deficient mice are used in most experimental studies,\(^6\) other animal models such as rabbits\(^7\)\(^-\)\(^9\) and pigs\(^10\)\(^,\)\(^11\) have also been frequently used to study atherosclerosis. This article highlights some recent \textit{ATVB} publications that have either extended the traditional concepts or provided new insights into understanding mechanisms of atherosclerosis.

**Lipid and Lipoprotein Metabolisms in Atherosclerosis**

**LDL and High-Density Lipoprotein**

There is convincing evidence that high plasma LDL-cholesterol concentrations contribute to the initiation and the progression of atherosclerosis, and lowering this lipoprotein reduces atherosclerosis-related cardiovascular events.\(^5\)\(^,\)\(^12\)\(^-\)\(^14\) In contrast, plasma high-density lipoprotein (HDL) cholesterol concentrations are negatively associated with atherosclerosis.\(^5\)\(^,\)\(^13\) Although the major clinical use of statins is to reduce plasma LDL-cholesterol concentrations, this class of drug may also increase plasma HDL-cholesterol concentrations.\(^15\)\(^,\)\(^16\)

Both LDL and HDL particles are highly heterogeneous.\(^18\) Recent advances in research of lipoproteins have provided new insights from \(\geq 2\) aspects. On the one hand, using techniques to detect and characterize subclasses of LDL particles,\(^19\)\(^-\)\(^25\) small dense LDL particles have been demonstrated in recent human studies to be positively associated with coronary heart disease.\(^21\)\(^,\)\(^23\)\(^,\)\(^24\) On the other hand, raising plasma HDL-cholesterol concentrations had no apparent beneficial effects on atherosclerosis.\(^25\) One lesson learned from the failure of the latter study is that HDL function may play a more critical role in preventing and protecting against atherosclerosis.\(^26\)\(^,\)\(^27\)

Studies focusing on exploring mechanisms of HDL dysfunction showed that myeloperoxidase impaired effects of apoA-I on reverse cholesterol transport,\(^28\)\(^,\)\(^29\) scavenger receptor type BI played a crucial role in HDL regulation of hematopoietic stem/progenitor cell proliferation and differentiation,\(^30\) and anti-inflammatory effects of HDL in macrophages were mediated by activating transcription factor 3, a protein involved in toll-like receptor signaling pathway.\(^31\)\(^,\)\(^32\) Using lipid chromatography-mass spectrometry technique, small, dense HDL3 particles were found to be associated with multiple protective effects in atherosclerosis, such as cholesterol efflux, anti-inflammation, and antioxidation.\(^33\)

**ATP-Binding Cassette Subfamily A Member 1**

ATP-binding cassette subfamily A member 1 (ABCA1) in macrophages facilitates cellular cholesterol efflux. Previous studies determining effects of ABCA1 on atherosclerosis in mouse models have been consistent. Deficiency of ABCA1 alone, or in combination with deficiency of ABCG1, in leukocytes, as demonstrated by bone marrow transplantation, augmented atherosclerosis in mice.\(^34\)\(^-\)\(^38\) Conversely, overexpression of ABCA1 in macrophages reduced atherosclerosis.\(^39\) However, conflicting findings were reported recently in studies using a genetic conditional deletion approach rather than bone marrow transplantation.\(^40\) Cell-specific deficiency of ABCA1 was created using Cre–Lox recombination technique.\(^41\) ABCA1 floxed mice expressed Cre transgene under the control of either the LysM or albumin promoter to develop myeloid or hepatocyte–specific ABCA1 deficiency. In 1 study, depletion of ABCA1 in hepatocytes augmented atherosclerosis in aortic roots of apoE\(^-\)\(^-\) mice, whereas macrophage deficiency of ABCA1 did not influence atherosclerotic development in LDL receptor\(^-\) (LDLR\(^-\)) mice.\(^42\) A subsequent study confirmed that myeloid cell–specific deficiency of ABCA1 had no significant effects on atherosclerosis development although it resulted in profound cellular cholesterol accumulation in resident peritoneal macrophages.\(^43\) In contrast to its deficiency in apoE\(^-\)\(^-\) mice, hepatocyte–specific deficiency of ABCA1 in LDLR\(^-\) mice attenuated atherosclerosis in aortic roots but had no effect on atherosclerotic lesion size of the entire aorta.\(^44\) In LDLR\(^-\) mice with hepatocyte–specific deficiency of ABCA1 fed an atherogenic diet, both apoB-containing lipoproteins and HDL were reduced. In vitro experiment inferred that HDL concentrations per se were not the primary contributor to plasma efflux capacity.\(^44\) Liver X receptor regulates both ABCA1 and ABCG1 and contributes to cholesterol efflux. A recent study reported that activation of liver X receptor attenuated atherosclerosis, in the absence of both ABCA1 and ABCG1 in bone marrow–derived cells.
Proprotein Convertase Subtilisin/Kexin Type 9

Since its discovery, studies have identified functional mutations of proprotein convertase subtilisin/kexin type 9 (PCSK9) that are related to either hypercholesterolemia (gain-of-function mutations) or hypocholesterolemia (loss-of-function mutations) in humans, which are associated with increased and reduced cardiovascular risks, respectively.\(^{47-51}\) Findings in human studies have led to the development of PCSK9 inhibitors to reduce plasma cholesterol concentrations and risks for cardiovascular events.\(^{52}\) In addition to PCSK9, profiling of catenin atherosclerosis from a human biobank using microarray technique found increased PCSK6 (a PCSK family member apart from PCSK9) mRNA abundance in fibrous caps of symptomatic carotid atherosclerotic lesions.\(^{53}\)

In the past several decades, LDLR\(^{-/-}\) and apoE\(^{-/-}\) mice have been the most commonly used hypercholesterolemic mouse models to study mechanisms of atherosclerosis. Therefore, manipulations of genes of interest in mice have also been bred to generate LDLR\(^{-/-}\) or apoE\(^{-/-}\) background. Recently, 2 research groups have reported that delivery of either a human (D374Y) or a mouse (D377Y) mutation of PCSK9 expressed in adenoassociated virus by a single injection led to rapid and profound increases of plasma cholesterol concentrations in several mouse strains, and promoted atherosclerosis that was comparable with lesions developed in LDLR\(^{-/-}\) or apoE\(^{-/-}\) mice.\(^{54-57}\) In addition to providing a new hypercholesterolemic mouse model for atherosclerosis study, these 2 studies have also enhanced our understanding of PCSK9-mediated LDLR regulation, atherosclerosis, and related potential mechanisms.

Inflammation in Atherosclerosis

Inflammation is a critical contributor to the development of atherosclerosis. Accumulation of leukocytes, predominantly macrophages, is a prominent feature of atherosclerosis from initiation to advanced stages of evolution. Lipid accumulation in macrophages induces inflammation, and inflammation promotes and augments atherosclerotic development. Therefore, inflammation and atherosclerotic lesion development form a positive feedback loop. Benefitted from recent enhancements to techniques, mechanisms by which inflammation contributes to atherosclerosis is continuously providing new insights. These include determining the processes of macrophages in invading the arterial wall, trapping, polarization, and triggering a spectrum of inflammatory signals within atherosclerotic lesions.\(^{56-60}\)

Leukocyte-Specific Effects

Since the initial experiment using bone marrow transplantation approach to studying atherosclerosis in mice,\(^{61}\) this method has been used frequently by researchers to determine effects of genes of interest in leukocytes in the development of atherosclerosis in mouse models. Recently, using this technique, it has been demonstrated that cathepsin C deficiency,\(^{62}\) CD43 (an integral membrane glycoprotein),\(^{63}\) CC chemokine ligand 3,\(^{64}\) or angiotensin-convverting enzyme (a critical enzyme to generate angiotensin II)\(^{65}\) in leukocytes led to reductions of atherosclerosis in hypercholesterolemic mice at a single time point. In mice studied at multiple durations of Western diet feeding, deficiency of the common β subunit of the granulocyte macrophage colony-stimulating factor/interleukin-3 receptor in leukocytes only reduced lesion size transiently.\(^{66}\) In contrast to these components that reduced atherosclerosis, acyl-CoA: cholesterol acyltransferase 1 deficiency in leukocytes augmented atherosclerosis.\(^{67}\)

Another commonly used approach to determining macrophase-specific effects of genes of interest is to use conditional knockout mice developed with LysM Cre–Lox recombination technique.\(^{68}\) Using this approach with mice expressing LysM Cre recombinase, Basu et al\(^{69}\) found that genetic depletion of ribosomal protein L13a in macrophages augmented atherosclerosis in mice.

Neutrophils

Neutrophils are the largest population of leukocytes in blood. Although neutrophils play a rapid and critical role in acute inflammatory response, the contribution of this cell type to atherosclerosis has not been drawn much attention until recently.\(^{70-72}\) Hypercholesterolemia induced neutrophilia, which was associated with the initiation of atherosclerosis in apoE\(^{-/-}\) mice.\(^{70}\) Cathelicidin is a neutrophil granule protein. Genetic deficiency of cathelicidin led to reductions of atherosclerosis in apoE\(^{-/-}\) mice.\(^{71}\) Activation of neutrophils leads to cell death and releasing neutrophil extracellular traps. A recent cross-sectional prospective clinical study reported that plasma neutrophil extracellular traps were positively associated with coronary artery disease.\(^{72}\) Neutrophil gelatinase-associated lipocalin is a neutrophil granular glycoprotein. A human prospectively investigation found that this glycoprotein was positively associated with cardiovascular events including atherosclerotic disease.\(^{73}\) These 2 recent human studies provide clinical evidence that neutrophils may play an important role in the development of atherosclerosis.\(^{74}\)

Chemokines

The presence and activation of multiple chemokines in the development of atherosclerosis is a commonly described feature of atherosclerosis.\(^{75}\) Many chemokine–chemokine receptor interactions such as CCL2–CCR2, CX3C–chemokine ligand 1 (CX3CL1)–CX3C–chemokine receptor 1 (CX3CR1), CCL5–CCR5, and CCL19/CCL21–CCR7 contribute to atherosclerotic development.\(^{76}\) A pharmacological inhibitor of CX3CR1 reduced atherosclerosis in both LDLR\(^{-/-}\) and apoE\(^{-/-}\) mice, implicating that this chemokine receptor might be a potential target for treatment of atherosclerosis-related inflammation.\(^{77}\) Important roles of chemokine/chemokine receptor interaction were also noticed in human studies. For example, CCL19/CCL21–CCR7 axis were associated with risk for coronary artery disease in a Chinese population.\(^{78}\)
Resident Cell Types in Atherosclerosis

Endothelial Cell–Specific Effects

Endothelial dysfunction of the arterial wall is an initial step to provoke monocyte adhesion. This is followed by macrophage intruding the subendothelial area to form foam cells. Hypercholesterolemia is a critical contributor to endothelial dysfunction. As determined in hypercholesterolemic mice with several genetic manipulations, endothelial dysfunction after prolonged hypercholesterolemia was the result of both impairment of sensitivity to nitric oxide and reduced nitric oxide synthase cofactor bioavailability. In a gene expression analysis of mouse aortic endothelium in response to hypercholesterolemia, inflammation, or aging, 14 genes were significantly different between these different atherogenic stimulations and normal condition. Angiopoietin-like protein 2 (Angptl2), a proinflammatory protein, is abundant in endothelial cells. Findings using whole body genetic deficiency of Angptl2, bone marrow transplantation, and endothelial-specific overexpression of Angptl2, demonstrated that endothelial cell–derived Angptl2 contributed to atherosclerotic lesion formation, attributed to endothelial dysfunction. Krüppel-like factor 2, a member in zinc finger transcription factors, plays an important role in regulating endothelial function. LDL inhibited endothelial Krüppel-like factor 2 through regulation of DNA and histone methylation. This was proposed as one mechanism by which LDL induced endothelial dysfunction. Type 1 insulin-like growth factor–I receptor is a negative regulator of nitric oxide bioavailability in endothelium. Incomplete deletion of insulin-like growth factor–I receptor (insulin-like growth factor–I receptorα) led to accelerated endothelium regeneration in mice, implicating potentially enhanced endothelial repair. Bone morphogenic protein (BMP) 4 interacts with its receptors BMPRI I or BMPRI II for its bioactive actions. Stimulations, such as hypercholesterolemia, disturbed blood flow, or inflammatory responses, suppressed BMPRII in endothelium. Partial genetic deletion of BMPRII (BMPRII−/−) led to the augmentation of atherosclerosis in apoE−/− mice. In addition to evidence from experimental studies, endothelial dysfunction was associated with macrophage infiltration and neovascularization in patients with early stage coronary atherosclerosis.

Smooth Muscle Cell–Specific Effects

Vascular smooth muscle cells (SMCs) are the predominant cell type in the arterial wall. This cell type also plays a critical role in atherosclerosis development through multiple mechanisms. Recent studies explored mechanisms by which survival or apoptosis of this cell type influenced atherosclerosis. Wnt/β-catenin responsive gene, Wnt1-inducible secreted protein-1, was associated with suppression of mouse aortic SMC apoptosis induced by oxidative stress, and Wnt 1-inducible secreted protein-1 was lower in unstable, compared with stable, human atherosclerotic lesions. Inhibition of Akt1 (the serine–threonine kinase) interaction with forkhead class O transcription factor 3a contributed to survival of vascular SMCs in vitro. Transgenic mice with SMC-specific overexpression of Akt1 inhibited SMC apoptosis in atherosclerotic lesions without changing lesion size. Small proline-rich repeat protein 3 promoted cell survival and was more abundant in SMCs of human atherosclerotic lesions than that of normal arterial tissues. Genetic deficiency of small proline-rich repeat protein 3 augmented atherosclerosis in apoE−/− mice, which were attributed to regulation of Akt signaling in SMCs. Therefore, roles of SMC survival and apoptosis in the development of atherosclerosis through Akt signaling pathway have not been consistent in the literature.

New Emerging Mechanisms

MicroRNAs

Since the discovery of microRNAs (miRs) in 1993, this class of small noncoding RNAs has been recognized as comprehensive regulators to many physiological and pathophysiological conditions. Their contributions to atherosclerosis have also been studied in the past few years. Since its initial study in regulation of cholesterol homeostasis and subsequently in atherosclerosis development, miR-33 has become a most attractive target in miRs for drug development to treat dyslipidemia and atherosclerosis. Although initial studies showed that inhibition of miR-33 reduced atherosclerosis in LDLR−/− or apoE−/− mice, recent studies have reported that inhibition of this miR did not attenuate the progression of atherosclerosis in LDLR−/− mice, or its deficiency in bone marrow–derived cells did not reduce atherosclerotic lesions. In addition to miR-33, many other miRs contribute to the development of atherosclerosis through different mechanisms. For example, miR-24 regulates macrophage behavior. miR-126-5p promotes endothelial proliferation, miR-145 controls SMC differentiation, miR-155 contributes to inflammation, and miR-302a modulates cholesterol homeostasis.

Neuronal Guidance Cues

Neuronal guidance cues regulate neuronal migration and vascular patterning and development. Recent studies have identified several neuronal guidance cues as a potential mechanism of atherosclerosis, which added another exciting layer to the complexity of atherosclerosis mechanisms. Netrin-1 is a neuronal guidance cue that mediates chemorepulsion and chemotraction of axons. van Gils et al reported that netrin-1 and its chemorepulsive receptor UNC5b were present in macrophages of human atherosclerotic lesions. This molecule inhibited macrophage migration and was a chemottractant for SMCs. Netrin-1 deficiency in hematopoietic stem cells isolated from fetal liver reduced atherosclerosis in mice through multiple mechanisms including regulating inflammation. A subsequent study found that netrin-1 and UNC5b were increased in macrophages in vitro by incubation with oxidized LDL or inducers of oxidative stress. These effects were inhibited by hypoxia-inducible transcription factor-1α, implicating that hypoxia is a potential contributor to activation of netrin-1–UNC5b axis. Semaphorin 3E, another neuronal guidance molecule, has also been implicated in atherosclerosis development. This molecule was abundant in macrophages of mouse atherosclerotic lesions and was increased by stimulation with oxidized LDL or hypoxia. In addition to important roles of netrin-1
and semaphorin 3E on macrophage function, these 2 guidance cues and another molecule, ephrinB2, were expressed in arterial endothelial cells. Netrin-1 and semaphorin 3E inhibited chemokine-mediated monocyte migration and leukocyte adhesion, whereas ephrinB2 was a potent monocyte chemoattractant.124

Summary
Mechanistic studies during the past decades using in vitro systems, animal models, and human tissues have highlighted the complexity of pathophysiological processes of atherosclerosis. Hypercholesterolemia, as one of the major risk factors for the development and progression of atherosclerosis, is still the focus of many mechanistic studies and the major therapeutic target of atherosclerosis. Although there is a dire need to validate many experimental findings in humans, there is a large number of approaches that have been showing promise for contributing to future therapeutic strategies.

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None.

References


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In the article by Lu and Daugherty, which appeared in the March 2015 issue of the journal *Arterioscler Thromb Vasc Biol*. 2015;35:485–491. DOI: 10.1161/ATVBAHA.115.305380), a correction was needed.

In the section “Proprotein Convertase Subtilisin/Kexin Type 9,” the last 2 sentences of the first paragraph have been revised as follows to show that there was an increased expression of PCSK6, not PCSK9, in the fibrous caps of human atherosclerosis: “Findings in human studies have led to the development of PCSK9 inhibitors to reduce plasma cholesterol concentrations and risks for cardiovascular events.52 In addition to PCSK9, profiling of carotid atherosclerosis from a human biobank using microarray technique found increased PCSK6 (a PCSK family member apart from PCSK9) mRNA abundance in fibrous caps of symptomatic carotid atherosclerotic lesions.53” As a result, references 52 and 53 have been renumbered to be 53 and 52, respectively.

The authors apologize for the error.

The online version of the article has been corrected and is available at http://atvb.ahajournals.org/content/35/3/485.