Atherosclerosis contributes to major mortality and modality 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,5 other animal models such as rabbits7-9 and pigs10,11 have also been used frequently to study atherosclerosis. This article highlights some recent ATVB publications that have either extended the traditional concepts or provided new insights into understanding mechanisms of atherosclerosis.

Recent Highlights of ATVB

Atherosclerosis

Hong Lu, Alan Daugherty

LDL and High-Density Lipoprotein

LDL and High-Density 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.5,15 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.16,17

Both LDL and HDL particles are highly heterogeneous.18 Recent advances in research of lipoproteins have provided new insights from ≥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 B1 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, deletion 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.

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or myeloid cells, providing evidence that liver X receptor has ABCA1- and ABCG1-independent effects on the development of atherosclerosis.45,46

**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 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

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.58–64

**Leukocyte-Specific Effects**

Since the initial experiment using bone marrow transplantation approach to studying atherosclerosis in mice,64 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-converting 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 macrophage-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 al69 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.74 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.75 These 2 recent human studies provide clinical evidence that neutrophils may play an important role in the development of atherosclerosis.76

**Chemokines**

The presence and activation of multiple chemokines in the development of atherosclerosis is a commonly described feature of atherosclerosis.77 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.78 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.79 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.80
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,107,109 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,113 miR-145 controls SMC differentiation,114 miR-155 contributes to inflammation,116 and miR-302a modulates cholesterol homeostasis.119

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 chemotractant 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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Disclosures

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.