Abstract: Elevated levels of cholesteryl ester (CE)–enriched apoB containing plasma lipoproteins lead to increased foam cell formation, the first step in the development of atherosclerosis. Unregulated uptake of low-density lipoprotein cholesterol by circulating monocytes and other peripheral blood cells takes place through scavenger receptors and over time causes disruption in cellular cholesterol homeostasis. As lipoproteins are taken up, their CE core is hydrolyzed by liposomal lipases to generate free cholesterol (FC). FC can be either re-esterified and stored as CE droplets or shuttled to the plasma membrane for ATP-binding cassette transporter A1–mediated efflux. Because cholesterol is an essential component of all cellular membranes, some FC may be incorporated into microdomains or lipid rafts. These platforms are essential for receptor signaling and transduction, requiring rapid assembly and disassembly. ATP-binding cassette transporter A1 plays a major role in regulating microdomain cholesterol and is most efficient when lipid-poor apolipoprotein AI (apoAI) packages raft cholesterol into soluble particles that are eventually catabolized by the liver. If FC is not effluxed from the cell, it becomes esterified, CE droplets accumulate and microdomain cholesterol content becomes poorly regulated. This dysregulation leads to prolonged activation of immune cell signaling pathways, resulting in receptor oversensitization. The availability of apoAI or other amphipathic α-helix–rich apoproteins relieves the burden of excess microdomain cholesterol in immune cells allowing a reduction in immune cell proliferation and infiltration, thereby stimulating regression of foam cells in the artery. Therefore, cellular balance between FC and CE is essential for proper immune cell function and prevents chronic immune cell overstimulation and proliferation. (Circ Res. 2016;118:679-691. DOI: 10.1161/CIRCRESAHA.115.306246.)

Key Words: apolipoprotein A-I ■ atherosclerosis ■ cholesterol ■ inflammation ■ membrane microdomains
Feedback Regulation and Low-Density Lipoprotein Cholesterol Metabolism

The biological relationship between cholesterol and coronary heart disease (CHD) represents one of the most celebrated and fascinating stories in modern science. As early as 1910, it was recognized that cholesterol was present in human aortic plaques. The term atherosclerosis, from the Greek word atheros meaning gruel, describes the color and consistency of coronary plaques. In 1955, another major discovery showed a significant association between low-density lipoprotein (LDL) cholesterol and CHD. This along with other key findings led to the elucidation of the LDL receptor pathway, for which the 1985 Nobel prize was awarded to Goldstein and Brown. During the 1980s, inspired by this and other key findings, pharmaceutical companies synthesized and tested HMG-CoA reductase inhibitors to decrease cholesterol synthesis and increase LDL receptor function. As expected, the widespread use of these drugs has led to lower plasma LDL cholesterol and a reduction in heart attacks. An LDL-centric approach toward treating CHD remains viable today. The latest drugs and therapeutic interventions are focused on regulating newly discovered steps along the LDL receptor pathway, thereby, providing multifaceted control of plasma concentrations and reductions in CHD. In the early 1980s, although many focused on the lipid-related aspects of CHD, vascular biologists concentrated on characterizing the numbers and types of immune cells within the artery wall during lesion progression. Eventually, by the late 1990s, a unified concept of atherosclerosis as a chronic disorder having both lipid and immune cell origins was on its way to wide acceptance.

LDL Versus High-Density Lipoprotein Metabolism: Related but Decidedly Different

The mechanisms controlling plasma high-density lipoprotein (HDL) concentration were originally anticipated to follow a feedback pathway similar to that of LDL. However, nothing could be further from the truth, as with every milestone, HDL was found to be distinctly different from LDL. Despite these complexities, higher plasma HDL concentrations are statistically associated with reduced CHD. Yet, recent pharmacological approaches strongly indicate that the manner in which HDL levels are elevated is critical for efficacy. Epidemiological studies demonstrating statistical associations between plasma cholesterol concentrations and CHD indicate that LDL and HDL participate in opposite but equally significant ways. Largely driven by statistical associations with human disease, mechanisms describing the dynamic nature of HDL metabolism have been elucidated over the decades through the work of numerous laboratories. These discoveries include the identification of the important plasma HDL cholesterol-esterifying enzyme (lecithin:cholesterol acyltransferase), an HDL receptor (scavenger receptor type B1 [SR-B1]), an HDL-modifying protein (cholesterol ester [CE] transfer protein), and a cholesterol transporter that exports cellular cholesterol and phospholipid from the plasma membrane to apoAI yielding nascent HDL particles, ATP-binding cassette transporter A1 (ABCA1) to name only a few. These discoveries brought important new knowledge to the HDL field, suggesting that the role for HDL in mitigating the damaging effects of elevated plasma LDL was through a process termed reverse cholesterol transport. The reverse cholesterol transport concept has withstood the test of time but has been recently updated by elegant studies showing that cholesterol efflux capacity is an inherent property of an individual’s own plasma HDL. These studies have led to the realization that measures of HDL function are superior to HDL concentration in predicting CHD risk. Although our understanding of how HDL protects the artery wall from lipid deposition remains an enigma, numerous studies have demonstrated that HDL protects the artery from both cholesterol loading and immune cell infiltration, suggesting that cholesterol efflux is the key component to understanding HDL’s protective properties.

Why Cholesterol Matters?

It has been known for decades that macrophage foam cells are the hallmark of early atherosclerotic lesions in humans and animal models of atherosclerosis. Foam cells are defined as cells with numerous droplets of CE that are visualized using a neutral lipid stain, such as Oil red O. Why immune cells accumulate CE as intracellular inclusions seems to result from an imbalance in the rate of cholesterol uptake compared with the rate at which the cell can remove cholesterol via efflux mechanisms. Although immune cells, like most cells, can use intricate feedback mechanisms of cholesterol uptake and synthesis, they express numerous unregulated scavenger receptors. In plasma, very low-density lipoprotein, LDL, and HDL come in contact with circulating peripheral blood mononuclear cells, and through pinocytosis their cholesterol content reflects the levels of plasma LDL cholesterol as measured by the free to ester cholesterol content of peritoneal macrophages. Foam cell formation may occur from uptake of LDL via scavenger receptor(s) or pinocytosis, as depicted in Figure 1. When plasma LDL concentrations are high and HDL concentrations are low, peripheral blood mononuclear cells (yellow) become cholesterol enriched and may display...

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ABCA1</td>
<td>ATP-binding cassette transporter A1</td>
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<tr>
<td>ACAT</td>
<td>acyl-CoA:cholesterol acyltransferase</td>
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<tr>
<td>AD</td>
<td>Alzheimer disease</td>
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<tr>
<td>CE</td>
<td>cholesteryl ester</td>
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<td>CHD</td>
<td>coronary heart disease</td>
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<td>FC</td>
<td>free cholesterol</td>
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<td>Foxp3</td>
<td>forkhead Box P3</td>
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<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
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<tr>
<td>PI3K</td>
<td>phosphatidylinositol-3-OH kinase</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
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<tr>
<td>SR-BI</td>
<td>scavenger receptor type B1</td>
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<tr>
<td>TCR</td>
<td>T-cell receptor</td>
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<td>Th</td>
<td>T helper cell</td>
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<td>Tregs</td>
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signs of cholesterol dysregulation or imbalance. Cholesterol-enriched monocytes are more likely to adhere to damaged endothelium where they migrate into the intima of the artery and become macrophages (blue). These processes cause smooth muscle cell expansion, promoting diffuse intimal thickening. As this process continues, more immune cells are recruited into the intima where they proliferate causing increased infusion of lipoproteins that aggregate and become oxidized or enzymatically modified. The inset in Figure 1 shows how free cholesterol (FC) homeostasis is maintained. After taking up lipoproteins through scavenger receptors, lipoprotein cholesteryl ester (CE) is hydrolyzed in the lysosome to FC, which is then shunted to the endoplasmic reticulum (ER), re-esterified by acyl-CoA:cholesterol acyltransferase (ACAT), and finally stored in cytoplasmic lipid droplets. The storage of CE is reversible because neutral cholesterol ester hydrolases (nCEHs) can convert CE to FC as needed. Then, ATP-binding cassette transporter A1 (ABCA1) can transport excess FC to apoAI and apoE. If influx rates are excessive, FC cannot be efficiently esterified or effluxed to extracellular acceptors. A consequence is that lipid raft platforms are not properly disassembled, and immune cell activation and signaling remain high leading to chronic inflammatory states. ICR indicates immune cell receptor; and NKT, natural killer T cells.

If influx of LDL exceeds the cell’s ability to efflux cholesterol, then macrophages become foamy. Despite the associations between hypercholesterolemia, foam cell formation, and CE accumulation in the arterial intima, it has been difficult to unequivocally demonstrate that CE accumulation is inflammatory. In fact, accumulation of CE in lipid droplets has been viewed as a CE depot that is biologically inert, a protective response to increased FC levels. CE accumulation in immune
cells is a clear indication that the cell has accumulated more FC than it can safely maintain in its cellular membranes. This condition also reflects an imbalance in the rate of LDL influx compared with the cell’s ability to efflux FC to acceptors, such as HDL. It has also been shown that cholesterol loading in macrophages causes cholesterol crystal and nucleotide-binding domain, leucine-rich repeat family, pyrin domain containing protein-3 inflammasome formation followed by the generation of proinflammatory mediators, such as interleukin (IL)-1β. It is believed that the accumulation of cholesterol crystals and their resolubilization by external acceptors are indicative of inefficient or defective cholesterol esterification or efflux causing crystals to form under some circumstance while not under others. Thus, it seems that the abundance of scavenger receptors on immune cells necessitates the need for additional pathways to control cholesterol removal as a means of maintaining healthy cellular cholesterol balance.

Supporting this concept, a recent study of elicited mouse peritoneal macrophages from hypercholesterolemic mice found that foam cells displayed an anti-inflammatory phenotype. The in-depth lipodomic and transcriptomic analyses of this work revealed a phenotype with marked suppression of proinflammatory mediator secretion along with increased production and accumulation of desmosterol. This suggests that extrinsic proinflammatory factors must be involved within the artery wall to stimulate a proinflammatory foam cell program in vivo. These results seem counterintuitive and suggest that the use of stimulated elicited macrophages may not mimic the in vivo. These results seem counterintuitive and suggest that the use of stimulated elicited macrophages may not mimic the

Are Lipid Droplets Inert?
Lipid droplets are intracellular organelles specialized for the storage of neutral lipids. Extensive evidence shows that FC accumulation within cells is toxic, proinflammatory, and proatherogenic. Despite how cholesterol enters the cell, unless proper signals and acceptors are present to promote efflux from the cell, it will eventually become overloaded and accumulate CE droplets. Although their formation can be induced in any cell type, they are regularly found in tissues that store fat for specialized functions. Unlike FC accumulation, it has been believed that CE droplets are inherently inert. The process of converting excess FC to CE by ACAT is believed to represent a protective mechanism, averting the toxic effects of excess FC within the cell. In Alzheimer disease (AD), CE accumulation in the central nervous system is linked with neurodegeneration and inhibition of CE synthesis has been shown to reverse β-amyloid peptide accumulation. It has been shown that ACAT inhibition indirectly enhances the movement of the nascent amyloid precursor protein molecules into the early secretory pathway. In addition, genetic ablation of ACAT in AD mice diminished the levels of the AD marker, Aβ42, decreased the amyloid plaque burden of full-length human amyloid precursor protein, and improved cognitive function. A recent gene therapy study in AD mice showed that adeno-associated virus targeting of ACAT1 for gene knockdown decreased the levels of total brain amyloid-β oligomeric amyloid-β and full-length human amyloid precursor protein to levels similar to those measured in AD mice with complete genetic knockdown of ACAT1.

However, a growing body of evidence suggests that formation of CE-rich lipid droplets, or lipid bodies as they have been called in leukocytes, can be proinflammatory. In leukocytes, these CE-rich lipid bodies are metabolically active and consist of a core of neutral lipids surrounded by a phospholipid monolayer containing specific embedded proteins. Such as perilipin 2, adipose differentiation-related protein. Thus, lipid droplets should not be considered passive inert structures because it is known that the CE in these structures undergoes a continual cycle of hydrolysis and re-esterification, an essential process in releasing FC for membrane lipid raft maintenance and efflux. The lipid droplets in leukocytes and many other types of cells have functions, compositions, and structural aspects distinct from classic lipid droplets found in adipocytes. However, there are also common proteins found in lipid droplets of all cells and tissues, such as the ancient ubiquitous protein that plays a role in the ubiquitin-conjugating machinery involved in proteasome degradation of proteins, such as the cholesterol synthetic enzyme HMG-CoA reductase. Recent studies suggest that lipid bodies found in macrophages, neutrophils, and eosinophils are highly dynamic structures formed in response to a variety of inflammatory conditions and their presence can be used as markers of leukocyte activation. Leukocyte lipid-droplet formation has been observed after infection by hepatitis C, Trypanosoma cruzi, and exposure to various bacterial products. Work in eosinophils has shown that lipid bodies contain inflammatory and cell signaling mediators, such as prostaglandins. It remains to be shown whether blocking or reducing lipid body formation in leukocytes can change the course of disease progression. Therefore, critical questions remain as to the function of stored neutral lipids in cell signaling and leukocyte inflammation, beyond the simple storage of excess FC as an inert, neutral lipid.

Membrane Cholesterol and Lipid Raft Microdomains
Glycerophospholipids, sphingomyelin, and FC, but not CE, form regularly distributed highly ordered 5- to 500-nm-diameter structures called lipid rafts, microdomains, or nanodomains depending on their size. By definition, membrane rafts are small heterogeneous, highly dynamic, sterol-, and sphingolipid-enriched domains that compartmentalize cellular processes. These rafts are detergent-resistant membrane complexes rich in FC, where FC is believed to help stabilize the raft through hydrophobic binding to the other components. Two common types of lipid raft have been reported; one is the planar lipid raft and the other is the invaginated lipid raft or caveolae, the little cave, whose structure depends on the caveolin proteins that are unique to caveolae. Cholesterol is an essential component of both lipid rafts and caveolae. These structures generally contain 3 to 5x the amount of FC than the surrounding membranes and have been shown to organize and compartmentalize many different protein components. Both types of rafts are found within the outer leaflet of the plasma membrane and arise from cholesterol’s hydrophobic interaction with sphingomyelin and glycerophospholipids.
Many critical enzymes and signaling systems, for example, endothelial nitric oxide synthase, SR-B1, Ras, CD36, Rho, mitogen-activated protein kinase, G-protein–coupled receptors, Cα2 regulatory proteins, glycosylphosphatidylinositols, and phosphatidylinositol phosphates, are active when concentrated within these microstructures, modulating immune cell activation and function. It is believed that efficient signal transduction requires signaling molecules to be reorganized, sequestered, and compartmentalized into nanodomains at the plasma membrane. The unique lipid composition and structural rigidity of these cholesterol-rich domains allow compartmentalization through lipid–lipid, lipid–protein, and membrane–cytoskeletal interactions. Although lipid rafts are typically studied at the cell surface, microdomains can also be found in other cellular membranes, such as the Golgi, mitochondria, lysosomes, and lipid droplets. The importance of these domains for immune cell activation and polarization has been widely studied in many different systems using the addition of β-cyclodextrin or squalene directly in vivo or in vitro to deplete or replete membrane cholesterol. In particular, the role of lipid rafts in bone marrow stem cell hierarchy is consistent with these structures acting as the master regulators of hematopoietic stem cell retention and quiescence in bone marrow niches, as well as serving a role in regulating their mobilization and homing. Therefore, how the cell regulates lipid raft formation, composition, and disassembly is of great interest and could be used for therapeutic intervention in cardiovascular disease.

Foam cell formation occurs when the influx of cholesterol is not balanced with the outflow, that is, influx is greater than efflux resulting in the accumulation of CE droplets. Cells require FC for lipid raft maintenance; when that need is met, excess FC is stored in the cytoplasm as CE. Differences in the rate of influx versus efflux will shift the intracellular cholesterol balance to promote a progressive increase in foam cell cholesterol loading or loss of foam cell cholesterol. We propose that the measure of this process is the EC/total cholesterol ratio; a high value implies foam cell progression, whereas a low value implies foam cell regression. Cholesterol influx is promoted by scavenger receptors and the LDL receptor. Cholesterol efflux is promoted by ABCA1-mediated transport to apoAI for delivery to the liver. However, ABCA1 will also efflux cholesterol to other proteins that carry amphiphatic helices, such as apoAI, apoAIV, apoCII, apoCIII, and apoE. Immune cells synthesize apoE but not apoAI. Local synthesis of apoE and the participation of transfer proteins and lipases synthesized by the macrophage may play a significant role in effluxing FC. However, plasma contains generous amounts of mature HDL and smaller amounts (≈2%) of lipid-poor or pre–β-HDL that may serve as an acceptor for ABCA1. Because immune cells highly express scavenger receptors, it is possible that mature HDL is reorganized at the membrane surface through its interaction with SR-B1, another protein associated with caveolae, that removes the CE core, leaving lipid-poor apoAI that can then interact with ABCA1 to package FC, sphingomyelin, and glycerophospholipids into nascent HDL particles, as shown in Figure 2. Uptake, and possibly efflux, is believed to be mediated, in part, by the presence of an extracellular matrix protein called procollagen C-endopeptidase enhancer protein 2 that contains 2 CUB, complement C1r/C1s, Uegf, and Bmp1, and a C-terminal netrin-like domain. The netrin-like domain of procollagen C-endopeptidase enhancer protein 2 anchors the protein to heparin sulfate proteoglycans in the extracellular matrix, whereas the CUB domains bind to apoAI and enhance SR-BI–mediated uptake of CE (Figure 2). To have ABCA1-mediated FC efflux from the cell after SR-BI–mediated uptake of CE may seem to be a futile cycle. However, if the nascent HDL particles formed by ABCA1 carried more moles of cholesterol than the original, mature HDL particles, then there would be a net loss of FC. Transfer proteins and lecithin–cholesterol acyltransferase may ensure that FC and newly synthesized CE are diluted into plasma away from the cell. There is some justification for this speculation as the 2 principal species of mature HDL carry fewer moles of total cholesterol per apoAI than nascent HDL. The other lipid-transport protein that may play a role in this process is ABCG1 that facilitates lipid transport out of the macrophage but has not been shown to directly transfer cholesterol to apolipoproteins or lipoproteins at the surface of the cell.

**Microdomain Cholesterol Regulation: Role of Efflux**

Total body cholesterol is maintained by secretion of bile from the liver into the feces because cells do not catabolize FC and are only able to regulate its concentration by exporting FC to the plasma in the form of lipoproteins. Transport is accomplished by packaging sphingomyelin, FC, and glycerophospholipids with apoAI by ABCA1, an abundant plasma protein synthesized by the liver and intestine, that possesses detergent qualities within its highly amphipathic structure. Nascent HDL particles are synthesized by ABCA1-mediated transport of cholesterol, sphingomyelin, and glycerophospholipids to lipid-poor apoAI. After the nascent particle enters the plasma, it is rapidly modified by lecithin–cholesterol acyltransferase and other proteins/enzymes to become a mature HDL hav- ing a CE-rich core. Given the ubiquitous cellular presence of ABCA1, cholesterol efflux seems to be a continuous housekeeping function of all cells. However, unlike the liver and intestine, immune cells do not make apoAI. They must either use apoE, which is synthesized by many, if not all, peripheral tissues, including macrophages, to generate lipid-poor apoAI from mature HDL after CE removal by SR-BI, as shown in Figure 2. The reliance of the ABCA1-mediated pathway on lipid-poor apoAI would suggest that SR-BI may play a critical role in mature HDL remodeling in plasma to promote hematopoietic stem cell cholesterol efflux and prevent hematopoietic stem and progenitor cell proliferation–related atherosclerosis.

Given the importance of microdomains, also called nanodomains, in providing a platform for organizing the signaling of many receptors and proteins, including the B-cell receptor, T-cell receptor, and major histocompatibility class receptors, it follows that lipid raft composition must be carefully regulated. The cholesterol needed for lipid raft formation and maintenance can be derived from exogenous sources, such as lipoproteins,
especially LDL, or from cellular synthesis via the mevalonate pathway in the endoplasmic reticulum followed by transport to the plasma membrane.\textsuperscript{41,97} Other sources of cholesterol are intracellular lipid droplets. The movement of cholesterol out of lipid droplet relies on extrinsic signals promoting the hydrolysis of CE by cholesterol ester hydrolases.\textsuperscript{98,99} FC can be either used for cellular membrane maintenance or moved to a substrate pool for export via ABCA1.\textsuperscript{25,100} ABCA1 under the control of the liver X receptor is the uniquely sensitive master controller of membrane cholesterol that regulates lipid raft composition.\textsuperscript{27} ABCA1 was originally discovered while investigating the molecular defect in individuals with Tangier disease who lacked normal levels of plasma HDL.\textsuperscript{101} It was quickly realized that the cholesterol transport function of ABCA1 was essential in maintaining lipid raft composition and function.\textsuperscript{102–108} To remove cholesterol efficiently from the cell, ABCA1 requires the assistance of proteins that solubilize and organize hydrophobic cholesterol molecules into lipoprotein particles and target them to the liver for elimination.\textsuperscript{109} One such protein, apoAI, the main protein constituent of HDL,\textsuperscript{110} is uniquely capable of these functions and is also one of the most abundant proteins present in plasma\textsuperscript{3} and lymph.\textsuperscript{111} Further recognition that cholesterol efflux and lipid raft cholesterol maintenance were one and the same process followed after discovering that the lipid composition of nascent HDL was similar to that typically found in microdomains from cell membranes.\textsuperscript{18,80} Figure 3 shows a mechanism by which ABCA1 mediates removal of FC from lipid rafts to form nascent HDL.\textsuperscript{18} Synthesis of nascent HDL containing raft cholesterol from 3 molecules of lipid-poor apoAI yields a \( \approx \) 9- to 11-nm-diameter particle that contains \( \approx \) 240 molecules of lipid; of which, 100 are FC.\textsuperscript{80} ApoAI along with sphingomyelin and glycerophosphatidylcholine solubilizes large amounts of FC that are transported to the liver for elimination. The contribution of immune cell FC to the total plasma cholesterol is small. However, the site of removal, the arterial atheroma, reduces lipid accumulation and the potential for arterial foam cell formation. Subcutaneous injection of microgram amounts of lipid-free apoAI into mice has resulted in improved immune cell function and reduced atherosclerosis while not appreciably changing the plasma HDL cholesterol concentration.\textsuperscript{112,113} These results demonstrate that critical changes in cholesterol concentrations associated with the artery may be accomplished with moderate or no change in the total plasma HDL cholesterol concentration. Likely apoE synthesized by immune cells, and to a somewhat lesser extent apoCs, also contributes to the process.

### Cholesterol and Immune Cell Proliferation and Migration

Net accumulation of immune cells into plaques is proportional to monocyte recruitment from bone marrow and local
proliferation within the plaque that is counterbalanced by the emigration and death of macrophages. A new focus on this process has examined how cholesterol loading of macrophages increased the expression of netrin 1 and semaphorin 3E that inhibit migration causing retention of macrophages in atherosclerotic lesions, a process that involves lipid-raft microdomains where the semaphorin receptor is located. Activation and proliferation of immune cells drive atherosclerosis and are characterized in hypercholesterolemic animal models by a sharp increase in the number of Ly-6Chi monocytes in circulation that are recruited to plaques. Cholesterol accumulation by monocytes significantly affects the recruitment and proliferation of these inflammatory monocytes, suggesting that uptake of modified LDL markedly impairs intracellular regulation of lymphocyte cholesterol. The process by which immune cells respond to alterations in cholesterol homeostasis has been extensively studied in hematopoietic stem and progenitor cells in bone marrow, which gives rise to monocytes and neutrophils. Hematopoietic stem and progenitor cells from both apoE−/− mice and ABCA1−/−, ABCG1−/− mice display increased cell surface levels of the common β-subunit of the granulocyte–macrophage colony-stimulating factor and IL-3 receptors because of increased cellular cholesterol concentrations. Bone marrow hematopoietic stem and progenitor cell expansion in response to hypercholesterolemia leads to neutrophilia and monocytosis, the latter leading to more inflammatory monocytes that are likely to infiltrate and remain in the artery wall. IL-3 and granulocyte–macrophage colony-stimulating factor contribute to leukocyte proliferation, differentiation, and survival and share a common β-chain receptor subunit CD131. IL-3 binds to the heterodimer IL-3 receptor α-chain, CD123, and CD131 leading to excessive leukocyte proliferation and fueling a cytokine storm that has recently been linked to atherosclerosis, exacerbation of myocardial infarction, heart failure, and sepsis. Studies have shown that CD131 localizes to lipid rafts when stimulated with granulocyte–macrophage colony-stimulating factor leading to activation of p38 mitogen-activated protein kinases, which activates cytokine gene expression. Increased cholesterol content of the outer leaflet of the macrophage plasma membrane was associated with increased Rac1 signaling, activation, and a decrease in chemotaxis, suggesting that both ABCA1 and ABCG1 are involved in regulating the microdomains in these cells. The central role of lipid microdomains in cell signaling and the constant necessity for regulating expansion and contraction of these microdomains attest to the importance of therapies that focus on regulating cholesterol homeostasis. Although the use of statins can reduce plasma cholesterol levels, regulation...
of cellular lipid microdomains requires cholesterol efflux via apoAI- or apoE-mediated pathways.

**T-Cell Activation and T Regulatory Cells: Role of Microdomains**

T cells can be found in the adventitial layer of normal, noninflamed arteries, but T-cell receptor (TCR)-αβ⁺ CD4⁺ cells can also be found in intimal plaques along with recruited monocytes during atherosclerosis progression. Recent studies have shown the importance of cellular cholesterol homeostasis in regulating T-cell proliferation through modulation of liver X receptor-β where oxysterol levels are reduced by SULT2B1. Like monocytes, the evidence that cholesterol-rich microdomains regulate T-cell function is incontrovertible. As early as 1998, investigators had discovered that T-cell lipid raft integrity played a role in TCR activation and diverted cells toward an inflammatory response. Further studies showed that CD28 engagement led to the redistribution and clustering of membrane and intracellular kinase-rich raft microdomains at the site of TCR engagements. In addition, recent studies show that distinct expression patterns of gangliosides were involved in the formation of lipid rafts in mature T cells, which affect both their differentiation and activation.

Because atherosclerosis is a dynamic process, the participation of T cells varies with the degree of inflammation. Several types of T helper cells participate in the inflammatory process; the proinflammatory T helper cell 1 (Th1) response is characterized by interferon-γ and tumor necrosis factor-β production, whereas the anti-inflammatory Th2 response produces IL-4 and IL-10. A balance between Th1 and Th2 responses is largely controlled by T regulatory cells (Tregs) that are critical in maintaining immunologic tolerance. Abundant evidence shows that Tregs are atheroprotective and that the normal Treg phenotype and function are disturbed during atherosclerosis progression. Recently, Lichtman’s group reported that the number of Tregs was reduced in aorta of atherogenic-diet fed mice yet reappear on regression of atherosclerotic lesions, suggesting an important functional role for these cells in atherogenesis. Subsets of Tregs include natural Tregs that develop in the thymus and express CD4⁺, CD25⁺, and the transcription factor forkhead box P3 (Foxp3), a key transcription factor regulating the differentiation and function of Tregs. In mice and humans with Foxp3 mutations, multiorgan autoimmune disease occurs, which is called scurfy in mice and immunodysregulation, polyendocrinopathy, enteropathy X-linked syndrome or IPEX in humans. Subsets of Foxp3⁺ Tregs that are generated within the periphery from CD4⁺ naïve cells are called induced or adaptive Tregs and are induced by a combination of TCR stimulation and transforming growth factor-β. This change converts CD4⁺CD25⁺Foxp3⁺ Th1 cells to CD4⁺CD25⁻Foxp3⁺ Th3 cells. These Treg subsets exert suppressive functions on other T cells through IL-10 and transforming growth factor-β production; both of which have been shown to influence atherogenesis. Lipid raft integrity was recently found to stabilize Foxp3 mRNA levels. Although in other studies, the role of mammalian target of rapamycin complex 1 showed that immunologic signals from the TCR to lipogenic pathways via sterol regulatory element-binding protein 1 directly influenced many cellular cholesterol and lipid biosynthesis pathways. Most importantly, with regard to Treg function and atherosclerosis, recent studies show that the lineage stability of Treg-suppressing cells is largely dependent on the activity of phosphatidylinositol-3-OH kinase (PI3K). Activation of signaling through PI3K and mammalian target of rapamycin suggests that Tregs require a mechanism for controlling PI3K distinct from that used by effector T cells. Consistent with this, PI3K inhibited the differentiation of homeostasis of Treg, whereas rapamycin promoted the proliferation and accumulation of Treg cells in the periphery. Inhibition of PI3K signaling enhances Treg cell differentiation and expression of Akt leading to an overall damping of the Treg-cell gene signature, including reduced expression of Foxp3, CD25, and cytotoxic T-lymphocyte-associated protein 4, a surface receptor involved in Treg function. Therefore, the role of lipid rafts in regulating Treg formation, stability, and function is yet another example of how unregulated membrane cholesterol influences the progression of atherosclerosis.

**Autoimmunity and Cholesterol Homeostasis**

In some respects, atherosclerosis has many similarities to an autoimmune disorder, as evidenced by the presence of self-antigens, such as heat shock protein 60 and LDL apoB, during atherosclerosis progression. Remarkable advances have enhanced our understanding of how innate immunity informs adaptive responses in atherosclerosis. For example, accelerated atherosclerosis is observed in individuals with systemic lupus erythematosus (SLE), a chronic autoimmune disease with a wide spectrum of clinical manifestations. It is characterized by overproduction of autoantibodies and increased B- and T-cell proliferation in lymph nodes and spleen. Continued autoantibody production eventually leads to kidney failure. However, of those who manage their SLE and do not die prematurely, the risk of CHD becomes great. The cause and pathogenesis of atherosclerosis are multifactorial, and the increased rates of CHD in patients with SLE are only partly explained by increased levels of traditional risk factors or from the drug therapy used to control the disease. In T cells from patients with SLE, cytotoxic T-lymphocyte-associated protein 4, a critical gatekeeper of T-cell activation, proliferation, and immunologic tolerance, was excluded from microdomains after CD3/CD28 costimulation. These results suggest that the inability of cytotoxic T-lymphocyte-associated protein 4 to localize in microdomains may explain why phosphorylation of proximal signaling molecules and proliferation were unchecked in these T cells.

Another chronic inflammatory disorder with autoimmune-like origins is rheumatoid arthritis. Individuals with rheumatoid arthritis also show exacerbated atherosclerosis progression. Although several factors contribute independently to the heightened cardiovascular risk observed in patients with either rheumatoid arthritis or SLE, systemic inflammation is likely a significant contributor to the process. In addition, patients with autoimmune disorders typically have higher levels of LDL and lower HDL levels when compared with control patients. Thus, the link between autoimmunity,
atherosclerosis, and the protective effects of HDL apoAI is clearly a topic of great clinical significance. The effects of HDL on atherosclerosis are widely accepted, but the HDL’s effect on immunity is not well understood. Recent studies with LDL receptor−/− mice lacking plasma apoAI showed that these mice had an increased susceptibility to the development of an autoimmune phenotype, characterized by enlarged lymph nodes, increased number of activated T cells, and autoantibody production. Interestingly, this phenotype seems to be triggered by ingestion of a cholesterol-rich diet and completely reversed by treatment with subcutaneous injections of apoAI at dosages that do not increase the concentration of plasma HDL apoAI. At the end of the infusion study there was an increase in Treg-cell number and a decrease in the percentage of effector/effector memory T cells suggesting that HDL apoAI is important for maintaining optimal Teff:Treg balance, that is, the balance between T helper cells that secrete cytokines and Tregs. Therefore, treatment of hypercholesterolemic mice with apoAI reduced both inflammation and the autoimmune phenotype. In other studies, recombinant HDL was shown to effectively stop inflammation in a mouse model of rheumatoid arthritis through its effects on dendritic cells, preventing nuclear translocation of nuclear factor κB. The most efficient means of cholesterol elimination is via the reverse cholesterol transport pathway. Since lipid rafts contain hydrophobic molecules and with the assistance of phospholipids form nascent lipoprotein particles, referred to as nascent HDL. Once these nascent HDLs enter the plasma compartment, they are acted on by an extensive group of enzymes and proteins that modify the particles to become what we normally observe in plasma as a mature HDLs. In many ways, these mature particles resemble their nascent counterparts, but instead of containing FC, they contain CE that condenses into the core of the lipoprotein. These CE-rich particles are removed from plasma by receptors on the liver for excretion, completing the reverse cholesterol transport pathway. Since lipid rafts contain high amounts of cholesterol and their composition is essential for preserving immune cell function, it follows that removal and solubilization of raft cholesterol via apoproteins protects immune cell function and accounts for a significant portion of the antiatherogenic and protective effects of apoAI on the vasculature.

**Concluding Remarks**

To preserve normal immune cell function, cholesterol homeostasis must be constantly monitored and maintained ensuring optimal lipid raft composition. Lipid rafts are unique cholesterol-rich microdomains that can be compared with platforms that compartmentalize or spatially organize proteins promoting kinetically favorable interactions for signal transduction or receptor activation. Conversely, microdomains may also separate signaling molecules, inhibiting interactions and dampening responses. Lipid rafts are found in all cellular membranes, including lysosomes and Golgi. Because all cells synthesize cholesterol but only the liver can catabolize it, elegant pathways to relocate and minimize the toxic effects of excess FC have evolved. One pathway converts FC to CE, forming droplets in the cytosol. With continued accumulation of CE, foam cell formation follows, one of the first indicators of atherosclerosis in humans. Using a simple measure of cellular CE/total cholesterol ratio provides a metric of the extent of foam cell progression. Thus, under conditions of high lipoprotein influx, cholesterol removal is essential for survival of the cell. The most efficient means of cholesterol elimination is via the membrane-bound transporter ABCA1 that moves intracellular cholesterol into contact with an apoprotein acceptor on the outer membrane surface. Because cholesterol is highly insoluble in aqueous solution, apoproteins solubilize these hydrophobic molecules and with the assistance of phospholipids form nascent lipoprotein particles, referred to as nascent HDL. Once these nascent HDLs enter the plasma compartment, they are acted on by an extensive group of enzymes and proteins that modify the particles to become what we normally observe in plasma as a mature HDLs. In many ways, these mature particles resemble their nascent counterparts, but instead of containing FC, they contain CE that condenses into the core of the lipoprotein. These CE-rich particles are removed from plasma by receptors on the liver for excretion, completing the reverse cholesterol transport pathway. Since lipid rafts contain high amounts of cholesterol and their composition is essential for preserving immune cell function, it follows that removal and solubilization of raft cholesterol via apoproteins protects immune cell function and accounts for a significant portion of the antiatherogenic and protective effects of apoAI on the vasculature.

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