ATVB in Focus
Novel Functions for HDL in Atherosclerosis and Immunity

Series Editors: Catherine Hedrick and Edward Fisher

Mary G. Sorci-Thomas, Michael J. Thomas

Abstract—This review provides a summary of recent research on the role of high-density lipoprotein (HDL)/apolipoprotein A-I cholesterol efflux and immune cell function. Plasma concentrations of HDL have been known to inversely correlate with risk for coronary vascular disease. Bulk transport of HDL cholesterol from the peripheral tissues to the liver is a major pathway, termed reverse cholesterol transport, responsible for maintaining whole body cholesterol homeostasis. In addition to participating in this pathway, HDL and apolipoprotein A-I exert anti-inflammatory effects through different pathways. One pathway that seems to be important in atherosclerosis and autoimmunity is its role in modulation of T cell activation. HDL/apolipoprotein A-I helps regulate cell signaling by accepting membrane cholesterol from ATP binding cassette transporter A1 on immune cells and, thereby, fine tuning the amount of cholesterol present in plasma membrane lipid rafts. (Arterioscler Thromb Vasc Biol. 2012;32:2561-2565.)

Key Words: apolipoprotein A-I  ■  atherosclerosis  ■  autoimmunity  ■  cholesterol efflux  ■  lipid rafts  ■  nascent high density lipoprotein  ■  T cells  ■  T regulatory cells (CD4+CD25+FoxP3)

Cholesterol Efflux and Nascent HDL Formation

The risk of developing coronary vascular disease (CVD) has been shown during many decades to be inversely correlated with plasma levels of high-density lipoprotein (HDL). However, recent studies suggest that HDL concentration does not always predict an individuals' CVD risk; rather, the amount of cholesterol efflux from cells seems a better predictor of CVD. Cholesterol efflux is the first step in the formation of HDL, which is initiated through the action of ATP binding cassette transporter (ABC) A1 on apolipoprotein (apo) A-I that produces nascent HDL (nHDL). Once nHDL enters plasma, the particles are extensively modified by a number of enzymes and transfer proteins to resemble what is referred to as mature HDL. The formation of nHDL is also the first step in the well-known reverse cholesterol transport pathway believed to be a major mechanistic basis for the protective effect of HDL. The majority of mature plasma HDL is derived from hepatic ABCA1 expression, although many other cells and tissues contribute a lesser extent. Formation of nHDL, and thus the cholesterol efflux, represents a continuous effort by cells to maintain their cholesterol content. One of the most important cellular cholesterol pools that the cell must maintain are the membrane microdomains known as lipid rafts. Given the significant inflammatory component of human atherosclerosis, maintenance of immune cell raft cholesterol composition is one of the most important aspects of future drug discovery targets in their search to control the progression of CVD.

Membrane Cholesterol Homeostasis and Lipid Rafts

A review of cellular control of cholesterol concentrations shows that there are 2 principal paths for accumulation of cellular cholesterol, namely synthesis and uptake of low-density lipoproteins (LDL) by the LDL receptor (LDLR), although the main pathway for removing cholesterol is by efflux to apoA-I forming HDL. Thus, cellular cholesterol levels are balanced by regulating the interplay between uptake, synthesis, and efflux. Lipids are removed from cells by several different ATP-dependent transporters; however, ABCA1 and ABCG1 seem to move the bulk of plasma membrane free cholesterol (FC) to lipid-free apoA-I and HDL, respectively. The apoA-I/ABCA1-mediated pathway for removal of cellular FC is a major source of plasma FC and plays an essential role in clearing excess cholesterol from cell. Cellular FC levels help control FC efflux. Sterol efflux by ABCA1 and ABCG1 is controlled by liver X receptors and retinoid X receptors upregulating the transcription of the genes encoding these transporters. Liver X receptors increase transcription after binding oxysterols synthesized by oxidation of FC. Curiously, some hydroxylases, such as CYP7A1 and B1, are under the control of liver X receptors. Regulation of apoA-I synthesis is more complex, but may also involve liver X receptors.

Cholesterol efflux through ABCA1 results in the biogenesis of nHDL particles and may be necessary for apoptotic cell engulfment. A variety of cell types, including macrophages, form similar-sized nHDL particles when incubated with lipid-free apoA-I. There has been considerable...
interest in determining the structure and composition of nHDL.\textsuperscript{30,32,33} In a comprehensive study of nHDL lipid composition, 10- to 12-nm nHDL particles derived from either mouse bone marrow-derived macrophages or from HEK293 cells were found to have similar ratios of FC to sphingomyelin (SM).\textsuperscript{33} Furthermore, these nHDL particles were found to be structurally organized with 3 molecules of apoA-I and containing ≈240 molecules of total lipid with the following composition: ≈43% FC, 37% glycerophosphocholine, and ≈10% SM.\textsuperscript{33} Electron microscopy and chemical crosslinking/mass spectrometry showed that the majority of these particles were spheroideal. When compared with plasma HDL isolated from the plasma of lecithin-cholesterol acyltransferase-deficient patients, where the normal lecithin-cholesterol acyltransferase catalyzed conversion of plasma HDL to cholesteryl ester (CE) is absent, the FC content was similar to the 10- to 12-nm diameter nHDL.\textsuperscript{34,35} Furthermore, in vitro studies show that nHDL FC is converted into CE-containing HDL,\textsuperscript{32} and that small nHDL particles do not seem to go on to become large nHDL particles, suggesting that there is no precursor–product relationship between the different-sized nHDL particles. Therefore, each particle seems to be derived from a separate step(s) in the lipidation process.\textsuperscript{32}

A significant observation from the studies of nHDL composition\textsuperscript{33} was that (1) >95% of cholesterol carried by nHDL particles was FC and (2) that the overall lipid composition of nHDL particles was similar to plasma membrane lipid fractions that have been described as lipid rafts.\textsuperscript{36} Although ABCA1 does not seem to be physically located in lipid rafts,\textsuperscript{37,38} the composition of ABCA1-generated nHDL suggests that ABCA1 accepts lipids from regions of the plasma membrane that have a lipid raft-like composition. Figure). A consequence of transferring SM and FC to nHDL could be a reduction of raft-like regions in the plasma membrane. Because these are specialized membrane domains for cell signaling, changes in the fraction of lipid raft may affect immune cell responses. Recent studies have shown that both ABCA1 and ABCG1 transfer SM with ABCG1, showing a greater ability for transferring SM to the medium.\textsuperscript{39-41}

The change in protein conformation going from lipid-free apoA-I to lipidated apoA-I suggests that the opening of lipid-free apoA-I to accept lipid takes place in a concerted fashion that can be simplified to discrete steps each of which entails sequential opening of helical pairs of the 4-helix bundle. Therefore, it is interesting to speculate on a mechanism that might explain the continuity of nHDL particle size and composition regardless of the cell type from which they are derived. There may be 3 points in the lipidation process at which further lipidation is hindered and nHDL was released from ABCA1. Given that the particles contain 1, 2, or 3 molecules of apoA-I, these steps seem to involve the sequential addition of apoA-I molecules during the lipidation process, (Figure). If the next apoA-I is unavailable, it is not properly opened, or there is insufficient lipid, then the nHDL particle is released from the ABCA1/apoA-I complex. This process also suggests the possible assistance of accessory protein(s) or chaperones that may aid in the assembly of 3 apoA-I molecules at the membrane surface as well as in opening of lipid-poor apoA-Is for assembly of the larger, more lipid-rich particles. In plasma, 3-apoA-I nHDL would be rapidly converted to a mature HDL in which CE has replaced nearly all of the FC forming a hydrophobic CE core and transforming the particle into a sphere.

T Cell Function, Atherosclerosis, and Autoimmunity

The role of immune cells in atherosclerosis has transformed the study of the disease progression.\textsuperscript{42} Because most of the FC in cells is located in the plasma membrane,\textsuperscript{43} lipid rafts are regions of the plasma membrane that are particularly enriched in FC, SM, and gangliosides. It has been postulated that rafts act as floating platforms on the plasma membrane which function to promote protein association.\textsuperscript{11,44} Recent studies have modified the notion of the classical raft by suggesting that lipid rafts are smaller structures that are associated with fewer proteins. These smaller islands are able to diffuse more efficiently and then cluster as necessary to promote signaling.\textsuperscript{45,46} Because lipid rafts are specialized membrane domains for cell signaling, they play an important role in immune cell function. Increased levels of plasma membrane cholesterol have been reported to promote the inflammatory response of T cells\textsuperscript{47} by enhancing the inflammatory T helper response.\textsuperscript{48} Reduction of plasma membrane FC\textsuperscript{50-51} or SM has been correlated with the attenuation of inflammatory responses.\textsuperscript{49}

Disruption of cholesterol efflux can be used as a basis for interpreting the effects of deleting either apoA-I or ABCA1 on immune cell function.\textsuperscript{52-58} One study investigated immune cell cholesterol homeostasis in mice lacking both the LDLr (LDLr\textsuperscript{−/−}) and apoA-I (LDLr\textsuperscript{−/−}, apoA-I\textsuperscript{−/−}) mice. As expected, when LDLr\textsuperscript{−/−}, apoA-I\textsuperscript{−/−} (DKO) mice were fed an atherogenic diet for 12 weeks they developed increased atherosclerosis compared with diet-fed LDLr\textsuperscript{−/−} (SKO) controls. Unexpectedly, they also displayed an unusual expansion and activation of T cells in their skin draining lymph nodes, which eventually led to an autoimmune phenotype.\textsuperscript{53} Furthermore, when T and B cells were fluorescence-activated cell sorter sorted from the lymph nodes of diet-fed DKO lymph nodes, these cells were found to be loaded with CE as measured by mass spectrometry,\textsuperscript{55} whereas cells from diet-fed SKO mice were not. These studies showed for the first time that cells other than monocytes/macrophages do become cholesterol enriched, leading to their dysfunction and in this instance the development of an autoimmune phenotype.

Cholesterol enrichment seems to be the stimulus that initiated T cell activation and expansion, as this phenotype was resolved by subcutaneous injections of lipid-free apoA-I into mice having cholesterol-laden immune cells.\textsuperscript{53} These results demonstrated that low plasma concentrations of HDL apoA-I reduced inflammation at concentrations well below those usually associated with mass cholesterol transfer. Previous reviews and studies have suggested a relationship between autoimmunity and atherosclerosis.\textsuperscript{59-63} Immune system involvement was confirmed by several studies that show T cells may participate in the atherosclerotic process, as reviewed,\textsuperscript{64-67} and these authors suggest that an imbalance of T cell subtypes is an important factor that determines either pro- or anti-inflammatory outcome. Of the various T cell
subtypes, the T regulatory cell (Treg-CD4+CD25+FoxP3+) has an important role in retarding inflammatory autoimmune diseases, such as diabetes mellitus, rheumatoid arthritis, and systemic lupus erythematosus. Treg cells are reported to suppress atherosclerosis by inhibiting proinflammatory T cells. As an example, vaccination against Foxp3+ in mice promotes atherosclerosis by reducing the number of Foxp3+ Treg cells available to suppress the actions of activated T cells in the plaque.

HDL and Sphingosine-1-Phosphate
Another important role HDL plays in immune cell activation relates to its role in transporting lipid mediators of immunity, such as sphingosine-1-phosphate (S1P). Several studies suggest that S1P may be a basis of the cardiovascular effects of HDL. Recent studies have shown that HDL is the primary carrier of apoM-bound S1P, a lipid mediator that has anti-inflammatory actions at low concentrations. Efflux of S1P from cells to plasma has been suggested to require transporters like ABCA1, and it is interesting to speculate whether apoA-I is an intermediary in the migration of S1P to apoM. S1P has been reported to promote the development of inflammatory T helper 1 cells while suppressing differentiation of Treg cells in contrast to its anti-inflammatory effects. FTP (Fingolimod), a structural analog of sphingosine and immune modulator, becomes phosphorylated by sphingosine kinase and then acts as a potent agonist at the 5 sphingosine-1-phosphate (S1P) receptors. FTY720 is now licensed (as Gilenya) for oral treatment of multiple sclerosis. There is a great deal to be learned regarding the role HDL plays in regulating S1P concentrations in plasma. A detailed understanding of the roles of S1P will require additional investigation.

Conclusions
Participation of apoA-I and HDL in reverse cholesterol transport has been well studied during the last half century, and plasma HDL concentration has been shown to have an inverse correlation with the risk for CVD. A more recent finding suggests that cholesterol efflux to HDL has a significant role in suppressing inflammation. HDL and apoA-I suppress inflammation by reducing the lipid-raft environment by promoting FC efflux from tissues and inflammatory cells, such as T cells and macrophages, resulting in an increased fraction of Treg cells that attenuate inflammation. Reduced levels of CE in inflammatory cells paralleled the reduction in FC. HDL carries S1P, a molecule that shows anti-inflammatory properties when present at lower concentrations. However, future studies will define the overall role of S1P in CVD and inflammation.

Sources of Funding
This research was supported in part by National Institutes of Health grants HL64163 and HL49373 to M.S.T.

Disclosures
None.

References


Sorci-Thomas and Thomas


High Density Lipoprotein Biogenesis, Cholesterol Efflux, and Immune Cell Function
Mary G. Sorci-Thomas and Michael J. Thomas

doi: 10.1161/ATVBAHA.112.300135

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/32/11/2561

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/