Role of HDL, ABCA1, and ABCG1 Transporters in Cholesterol Efflux and Immune Responses

Laurent Yvan-Charvet, Nan Wang, Alan R. Tall

Abstract—Atherosclerosis has been characterized as a chronic inflammatory response to cholesterol deposition in arteries, but the mechanisms linking cholesterol accumulation in macrophage foam cells to inflammation are poorly understood. Macrophage cholesterol efflux occurs at all stages of atherogenesis and protects cells from free cholesterol and oxysterol-induced toxicity. The ATP-binding cassette transporters ABCA1 and ABCG1 are responsible for the major part of macrophage cholesterol efflux to serum or HDL in macrophage foam cells, but other less efficient pathways such as passive efflux are also involved. Recent studies have shown that the sterol efflux activities of ABCA1 and ABCG1 modulate macrophage expression of inflammatory cytokines and chemokines as well as lymphocyte proliferative responses. In macrophages, transporter deficiency causes increased signaling via various Toll-like receptors including TLR4. These studies have shown that the traditional roles of HDL and ABC transporters in cholesterol efflux and reverse cholesterol transport are mechanistically linked to antiinflammatory and immunosuppressive functions of HDL. The underlying mechanisms may involve modulation of sterol levels and lipid organization in cell membranes. (Arterioscler Thromb Vasc Biol. 2009;29:00-00.)

Key Words: ABC transporter • apoptosis • immune system • lipids • cholesterol • inflammation

The removal of excess cholesterol from macrophage foam cells by HDL and its principal apolipoprotein, apoA-I, is thought to be one of the key mechanisms underlying the atheroprotective properties of HDL.1,2 Cholesterol accumulation in macrophage foam cells during atherogenesis induces inflammatory responses, apoptosis, and other adverse effects.3 Accumulating evidence suggests that by promoting cholesterol and oxysterol efflux, HDL regulates all these cellular responses in macrophage foam cells.4 ATP-binding cassette transporters ABCA1 and ABCG1 play a pivotal role in cholesterol efflux from macrophage foam cells. ABCA1 and ABCG1 show additive activity in promoting macrophage reverse cholesterol transport in vivo,5 and combined deficiency of these transporters in bone marrow–derived hematopoietic cells leads to severe defects in cholesterol efflux to HDL, massive cholesteryl ester accumulation in macrophages, and accelerated atherogenesis in a susceptible background.6,7 In addition, by modulating cholesterol homeostasis, ABCA1 and ABCG1 may be central to the antiapoptotic and antiinflammatory effects of HDL.4,5,8,9 Cholesterol accumulation in the plasma membrane of Abca1−/− and Abcg1−/− macrophages has been shown to increase signaling of Toll-like receptors enhancing the inflammatory response to LPS or other TLR ligands.10–13 As a consequence, mice lacking ABCA1 and ABCG1 accumulate prominent macrophage foam cells in various tissues such as in the lung, liver, spleen, or thymus,6,14–17 and in response to an inflammatory stimulus Abcg1−/− bone marrow transplanted mice revealed a profound inflammatory infiltrate in the adventitia and necrotic core region of atherosclerotic lesions.15

ABC Transporters and Active Cholesterol Efflux

Although passive cholesterol diffusion accounts for a large part of the efflux of cholesterol from nonloaded macrophages, active cholesterol efflux from macrophage foam cells via ABCA1 and ABCG1 represents as much as 70% of the total cellular cholesterol efflux following cholesterol loading.6,7,18,19 ABCA1 and Cholesterol Efflux to ApoA-I

Two distinct mechanisms have been proposed to explain ABCA1-mediated cholesterol efflux from macrophage to apoA-I. One is that apoA-I forms complexes with phospholipid and cholesterol at the cell surface in a process promoted by ABCA1 activity.20,21 There is abundant evidence that ABCA1-mediated cholesterol efflux to apoA-I can occur at the plasma membrane.22–24 The other is that apoA-I binds ABCA1 at the cell surface and is subsequently internalized and targeted to late endosomes, where apoA-I picks up lipids and the apolipoprotein-lipid complexes are then resecreted from the cell by exocytosis.25–27 Internalized ABCA1–apoA-I complexes have been localized within late endosomes. More decisively, a mutant version of ABCA1 containing a deletion

Received June 11, 2009; revision accepted September 15, 2009.
From the Division of Molecular Medicine, Department of Medicine, Columbia University, New York.
Correspondence to Laurent Yvan-Charvet, Division of Molecular Medicine, Department of Medicine, Columbia University, 630 W 168th St, New York, NY 10032. E-mail ly2159@columbia.edu
© 2009 American Heart Association, Inc.
Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.108.179283
of the PEST sequence within its cytoplasmic domain (PEST sequences are rich in proline, glutamic acid, serine, and threonine) showed defective internalization and trafficking to late endosome/lysosomes and defective cholesterol efflux to apoA-I after loading the late endosome/lysosome pool of cholesterol by AcLDL treatment. In a novel mode of posttranscriptional regulation, apoA-I stabilizes ABCA1 by preventing its calpain-mediated degradation in a PEST sequence-dependent fashion. A recent study by Lu et al helps to integrate these findings by showing that apoA-I stabilizes ABCA1 against calpain proteolysis after internalization of ABCA1. Although these mechanisms are not mutually exclusive, there is controversy as to which mechanism plays the dominant role in ABCA1-mediated cholesterol efflux from macrophages to apoA-I. Recent studies have confirmed internalization of apoA-I and its accumulation in the late endosome/lysosome compartments in ABCA1-expressing macrophages. These studies also showed that the majority of the rescared apoA-I had been degraded. However, these studies were performed using macrophages without cholesterol loading in the endosomal system. The mechanism governing the efflux after cholesterol loading, as pointed out by Oram and described above, are likely different. Indeed, a recent study has confirmed that the retroendocytosis of ABCA1 was critical in promoting cholesterol efflux through the ABCA1–apoA-I axis when excess lipoprotein-derived cholesterol had accumulated within cells. In contrast, this study also demonstrated that blocking retroendocytosis of ABCA1 did not affect cholesterol efflux from cells in the absence of cholesterol loading in agreement with previous studies.

### ABCG1 and Cholesterol Efflux to Mature HDL

Despite increasing interest and extensive study on the role of ABCG1, relatively little is known about how this transporter promotes efflux of cholesterol to mature HDL. Wang et al showed that ABCG1 promotes efflux of cholesterol to a variety of acceptors, including HDL, LDL, phospholipid vesicles, and cycloedextrin without increasing the binding of lipoproteins to cells. ABCG1 also promoted efflux of a broader spectrum of sterols including oxysterols such as 7-ketocholesterol, whereas ABCG1 and ABCG4 together promote efflux of sterol intermediates from the cholesterol synthesis pathway such as desmosterol. Interestingly, overexpression of ABCG1 increases efflux of not only cellular sterols but also, to some extent, cellular phospholipids. Overexpression of ABCG1 results in increased cell membrane cholesterol pools available for efflux and increases the rate constant for efflux; efflux appears to be diffusional and unidirectional and is more efficient for smaller HDL particles. Different models have been proposed to explain how ABCG1 promotes cholesterol efflux to HDL. One suggests that ABCG1 helps sterol molecules to overcome the energy barrier for entry into the hydrophilic water layer, perhaps by using ATP to promote protrusion of the cholesterol molecule into water, followed by a transient collision with an acceptor. A second model points to a function of ABCG1 as a phospholipid floppase, promoting changes in the organization of plasma membrane phospholipids and subsequent attraction of sterols to the outer leaflet for diffusional efflux. Another possibility is that ABCG1 promotes flopping of cholesterol in liquid ordered regions of plasma membrane such that spontaneous flipping of sterol back to the inner membrane is not possible.

### ABC Transporters and the Molecular Regulation of the Immune System

#### ABC Transporters and Macrophage Inflammation

HDL has potent antiinflammatory properties, including the ability to directly bind and sequester lipopolysaccharide, suppressing activation of Toll-like receptor 4 (TLR4) signaling. Recent studies have indicated additional direct cellular effects of HDL leading to suppression of inflammatory responses. By promoting cholesterol efflux, HDL has been proposed to inhibit cellular inflammatory signaling leading to inhibition of MCP-1 and CD11b expression and monocyte transmigration. Several studies have shown that the increased lipid raft formation in macrophages with genetic deficiencies of ABCA1 or ABCG1 could account for the enhanced inflammatory responses, especially after treatment with LPS or other TLR ligands leading to enhanced signaling via Myd88-NFkB (Figure). Replenishment or removal of cholesterol using cyclodextrin modulates the inflammatory response of macrophages deficient in ABCA1 or ABCG1, indicating that the increased inflammatory response is likely attributable to cholesterol accumulation in membranes (Figure). We recently reported that by modulating membrane cholesterol, deficiency of ABCA1 or ABCG1 increased TLR4 cell surface expression (Figure). ABCG1 appears to have a more potent role in modulating macrophage inflammatory response than ABCA1, perhaps reflecting a predominant role of this transporter in modulating the lipid composition of plasma membrane lipid rafts (liquid ordered domains). Interestingly, this response was not specific for TLR4, because macrophages lacking ABCA1 and ABCG1 were also more susceptible to TLR2 and TLR3 ligands. TLR3, in contrast to TLR2 and TLR4, is localized to the endosomal compartment. Recently, Sun et al showed that free cholesterol accumulation in the endosomal compartment increased the inflammatory response in a Toll-like receptor-dependent fashion, with TLR3 playing the major role. Together with the fact that ABCA1 plays a key role in removing cholesterol from the endosomal/lysosomal compartment, this suggests the hypothesis that there may be a unique role of ABCA1 in modulating inflammatory response initiated in late endosome/lysosomes (Figure).

### ABC Transporters and Lymphocyte Proliferation

Recently, Bensinger et al reported that Liver X Receptor (LXR) signaling coupled sterol metabolism to T-cell lymphocyte proliferation in an ABCG1-dependent fashion. ApoA-I KO mice also develop T-cell proliferation and activation and features of autoimmunity when backcrossed into an LDL receptor–deficient background. Together these studies strongly suggest that HDL-mediated cholesterol efflux via LXR-regulated ABC transporters plays a key role in down-regulating lymphocyte proliferation and activation. Bens-
ABCA1 and ABCG1 have complementary activities in mediating cholesterol efflux, and both transporters are LXR signaling events.

**Figure.** Protective effects of HDL and ABC transporters on macrophage inflammatory response induced by Toll-like receptors. HDL promotes cholesterol efflux via ABCA1 and ABCG1, and these transporters modulate the fluidity of the plasma membrane as well lipid raft formation. This, in turn, modulates the Toll-like receptor 4 signaling pathway by lipopolysaccharide (LPS) involving the formation of a LPS complex in lipid raft consisting of surface molecules such as CD14 and MD2. ABCG1 has a more potent role in modulating macrophage inflammatory response than ABCA1, but maximal effects require the activity of both transporters, suggesting complementary roles of these transporters. This response was not specific of TLR4 because deficiency of ABCA1 and ABCG1 also enhanced other Toll-like receptor signaling such as TLR2 present at the cell surface. Subsequently, downstream signaling molecule such as Myd88 will control the activation of NF-κB and the inflammatory gene expression response. Activation of LXR can modulate the inflammatory response through transregression of NF-κB, but this mechanism doesn’t seem to be controlled by ABCA1 or ABCG1 activity. Free cholesterol accumulation in the endosomal compartment also modulated the inflammatory response by spontaneous activation of Toll-like receptors present in this compartment such as TLR3. By promoting cholesterol efflux from the late endosome compartment, ABCA1 may have a unique role in dampening inflammation.
Summary and Future Directions

The studies of mice and cells with genetic deficiencies of ABCA1 and ABCG1 have helped to clarify the roles of these transporters in cholesterol and oxysterol efflux from macrophage foam cells. These processes have been shown to be linked to inflammatory and immune responses. In the future studies of mice with cell-specific knock-outs of transporters in mice of uniform genetic background may help to define the importance of the immune system effects in atherosclerosis and other inflammatory diseases of the lung and the underlying cellular mechanisms.

Disclosures

None.

References

Role of HDL, ABCA1, and ABCG1 Transporters in Cholesterol Efflux and Immune Responses
Laurent Yvan-Charvet, Nan Wang and Alan R. Tall

Arterioscler Thromb Vasc Biol. published online October 1, 2009;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 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/early/2009/10/01/ATVBAHA.108.179283.citation

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/