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

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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 atherosclerosis 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. 2010;30:139-143.)

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 atherosclerosis 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.4,8,9 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.13

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

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and the apolipoprotein-lipid complexes are then resecreted from the cell by exocytosis.\textsuperscript{25–27} Internalized ABCA1–apoA-I complexes have been localized within late endosomes. More decisively, a mutant version of ABCA1 containing a deletion 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.\textsuperscript{26} In a novel mode of posttranscriptional regulation, apoA-I stabilizes ABCA1 by preventing its calpain-mediated degradation in a PEST sequence dependent fashion.\textsuperscript{28,29} A recent study by Lu et al\textsuperscript{30} 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.\textsuperscript{30–32} These studies also showed that the majority of the resecreted apoA-I had been degraded.\textsuperscript{31} However, these studies were performed using macrophages without cholesterol loading in the endosomal system.\textsuperscript{30–32}

The mechanism governing the efflux after cholesterol loading, as pointed out by Oram\textsuperscript{13} 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.\textsuperscript{34} 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.\textsuperscript{30–32}

**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 cyclodextrin without increasing the binding of lipoproteins to cells.\textsuperscript{35} ABCG1 also promoted efflux of a broader spectrum of sterols including oxysterols such as 7-ketocholesterol,\textsuperscript{8} whereas ABCG1 and ABCG4 together promote efflux of sterol intermediates from the cholesterol synthesis pathway such as desmosterol.\textsuperscript{36} Interestingly, overexpression of ABCG1 increases efflux of not only cellular sterols but also, to some extent, cellular phospholipids.\textsuperscript{35,37,38} 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.\textsuperscript{39} 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 protonation of the cholesterol molecule into water, followed by a transient collision with an acceptor.\textsuperscript{40} A second model points to a function of ABCG1 as a phospholipid floppase,\textsuperscript{37} 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,\textsuperscript{41,42} suppressing activation of Toll-like receptor 4 (TLR4) signaling.\textsuperscript{43} 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.\textsuperscript{44} Several studies have shown that the increased lipid raft formation in macrophages with genetic deficiencies of ABCA1 or ABCG1\textsuperscript{10–13,45} could account for the enhanced inflammatory responses, especially after treatment with LPS or other TLR ligands leading to enhanced signaling via Myd88-NFkB (Figure).\textsuperscript{10–13,15} 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).\textsuperscript{12,13} We recently reported that by modulating membrane cholesterol, deficiency of ABCA1 or ABCG1 increased TLR4 cell surface expression (Figure).\textsuperscript{13} 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).\textsuperscript{38} 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.\textsuperscript{46} 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.\textsuperscript{47} ApoA-1 KO mice also develop T-cell proliferation and activation and features of autoimmunity when backcrossed into an LDL
ABCA1 and ABCG1 have complementary activities in mediating cholesterol efflux, and both transporters are LXR target genes. Therefore, the effects of single transporter deficiency could be masked by the compensatory upregulation of the other transporter as a result of sterol accumulation and LXR activation. Accordingly, in vivo measurements of macrophage reverse cholesterol transport have shown additive effects of ABCA1 and ABCG1, and bone marrow transplantation from mice with combined deficiencies of ABCA1 and ABCG1 into Ldlr−/− mice resulted in no change in atherosclerosis, most likely reflecting an unexpected decrease in VLDL and LDL levels in the double KO bone marrow transplant recipients. The latter study was interpreted as showing a disproportionate increase in atherosclerosis given the degree of VLDL/LDL lowering. We have not observed such decreases in VLDL/LDL in Abca1−/− Abcg1−/− bone marrow–transplanted mice, and the reasons for this discrepancy will require further studies. Interestingly, the Ldlr knockout recipient mice receiving bone marrow from Abca1−/− Abcg1−/− donor mice showed massive myocardial foam cell accumulation, as well as foam cell and inflammatory cell accumulation in other tissues such as intestine and spleen, most likely secondary to markedly increased overall and macrophage-specific inflammatory responses illustrating in vivo the relevance of ABCA1 and ABCG1 in dampening inflammation.

ABC Transporters and In Vivo Relevance of the Regulation of the Immune System: A Role in Atherosclerosis and Other Inflammatory Diseases

ABC Transporters and Atherosclerosis

Transplantation of bone marrow from Abca1−/− mice into Ldlr−/− or apoE−/− recipients caused an increase in atherosclerosis. However, conflicting data have been reported as deficiency of ABCG1 in bone marrow cells resulted in either a modest increase or decrease in atherosclerosis, and total body ABCG1 overexpression also resulted in either no effect, or protection against atherosclerosis. ABCA1 and ABCG1 have complementary activities in mediating cholesterol efflux, and both transporters are LXR target genes. Therefore, the effects of single transporter deficiency could be masked by the compensatory upregulation of the other transporter as a result of sterol accumulation and LXR activation. Accordingly, in vivo measurements of macrophage reverse cholesterol transport have shown additive effects of ABCA1 and ABCG1, and bone marrow transplantation from mice with combined deficiencies of ABCA1 and ABCG1 into Ldlr−/− mice resulted in no change in atherosclerosis, most likely reflecting an unexpected decrease in VLDL and LDL levels in the double KO bone marrow transplant recipients. The latter study was interpreted as showing a disproportionate increase in atherosclerosis given the degree of VLDL/LDL lowering. We have not observed such decreases in VLDL/LDL in Abca1−/− Abcg1−/− bone marrow–transplanted mice, and the reasons for this discrepancy will require further studies. Interestingly, the Ldlr knockout recipient mice receiving bone marrow from Abca1−/− Abcg1−/− donor mice showed massive myocardial foam cell accumulation, as well as foam cell and inflammatory cell accumulation in other tissues such as intestine and spleen, most likely secondary to markedly increased overall and macrophage-specific inflammatory responses illustrating in vivo the relevance of ABCA1 and ABCG1 in dampening inflammation.

ABC Transporters and Inflammatory Diseases

Mice lacking ABCA1 and ABCG1 accumulate inflammatory macrophage foam cells not only in the myocardium but also in various tissues such as in the lung, liver, spleen, or thymus. Several investigators have focused on the lung because mice lacking ABCA1, with low plasma HDL levels, revealed pulmonary lipidosis and progressive disease with chronic inflammation, and this was much more prominent...
in mice lacking ABCG1, with normal plasma HDL levels.6–14 The mechanism by which these transporters are involved in lung disease is thought to be similar to what has been described for atherosclerosis including enhanced Toll-like receptor-mediated macrophage inflammation.15,16 Consistent with this inflammatory phenotype, mice lacking ABCA1 and ABCG1 featured an inflammatory blood pattern characterized by increased neutrophils and monocytes.7,13 This suggests that by dampening inflammation, HDL may have a general beneficial effect on inflammatory diseases including atherosclerosis and perhaps some inflammatory pulmonary diseases. Overexpression of the human apoA-I transgene in mice resulted in lower cytokine levels and improved survival rates after LPS challenge compared to wild-type mice.60 whereas reconstituted HDL has been shown to reduce LPS-induced inflammation in rabbit, dogs, and humans.61–64 The beneficial effect of HDL is, at present, thought to be related to the LPS-neutralizing properties of HDL.41,42 These findings suggest that raising HDL may be a valuable therapeutic approach for a broad range of inflammatory diseases possibly including the treatment of septic shock and some inflammatory lung diseases.

**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.10–13,17,18,47,48 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.

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