Cell Metabolism
Review

HDL, ABC Transporters, and Cholesterol Efflux: Implications for the Treatment of Atherosclerosis

Alan R. Tall,[1] Laurent Yvan-Charvet,[1] Naoki Terasaka,[1] Tamara Pagler,[1] and Nan Wang[1,*]
[1]Division of Molecular Medicine, Department of Medicine, Columbia University, New York, NY 10032, USA
*Correspondence: nw30@columbia.edu
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High-density lipoprotein (HDL) has been identified as a potential target in the treatment of atherosclerotic vascular disease. The failure of torcetrapib, an inhibitor of cholesteryl ester transfer protein (CETP) that markedly increased HDL levels in a clinical trial, has called into doubt the efficacy of HDL elevation. Recent analysis suggests that failure may have been caused by off-target toxicity and that HDL is functional and promotes regression of atherosclerosis. New studies highlight the central importance of the ATP-binding cassette (ABC) transporters ABCA1 and ABCG1 in reducing macrophage foam cell formation, inflammation, and atherosclerosis. A variety of approaches to increasing HDL may eventually be successful in treating atherosclerosis.

While statins have revolutionized the treatment of atherosclerotic cardiovascular disease, they reduce cardiovascular events by about 20%–40% depending on the degree of low-density lipoprotein (LDL) reduction (Baigent et al., 2005; Kearney et al., 2008), leaving a large burden of residual untreated disease. There are extensive epidemiological data showing an inverse relationship between high-density lipoprotein (HDL) levels and coronary heart disease (CHD) (Castelli et al., 1977; Gordon et al., 1977; Lewington et al., 2007; Rhoads et al., 1976), and the HDL-raising properties of several lipid-lowering drugs, including statins, fibrates, bile acid sequestrants, and niacin, appear to be part of a spectrum of favorable effects (NHLBI, 1984; Brown et al., 2006; Otvos et al., 2006; Pedersen et al., 1998). Recent studies show that in patients treated with statins to lower LDL levels, HDL levels remain an independent predictor of the likelihood of suffering cardiovascular events (Barter et al., 2007a), and that in patients on statins with low LDL levels undergoing stent placement for acute coronary syndromes, HDL levels at baseline are a strong predictor of 12-month morbidity and mortality from cardiovascular events (Wolfram et al., 2006). Transgenic overexpression of apoA-I in mice and infusion of apoA-I/phospholipid complexes in humans cause reduced progression or regression of atherosclerosis (Nissen et al., 2003; Plump et al., 1994; Rubin et al., 1991). These observations have suggested that HDL-raising therapies might be an effective way to reduce the huge burden of residual cardiovascular disease in patients treated with current lipid-lowering therapies.

A Disappointing Therapeutic Failure Involving the CETP Inhibitor Torcetrapib

The hope for new HDL treatments was dealt a severe blow on December 2, 2006, when a large clinical trial called ILLUMINATE involving a potent HDL-raising drug, torcetrapib, was stopped prematurely as a result of an excess of deaths and morbidity from cardiovascular endpoints in the group receiving torcetrapib and atorvastatin compared to atorvastatin alone. Torcetrapib was at the forefront of a new class of drugs that potently raise HDL and lower LDL levels by inhibiting cholesteryl ester transfer protein (CETP) (Brousseau et al., 2004), a therapeutic concept that was first suggested by the dramatic high-HDL/low-LDL phenotype of human genetic CETP deficiency (Brown et al., 1989; Inazu et al., 1990). Unfortunately, torcetrapib also caused a moderate increase in blood pressure (2–5 mm systolic blood pressure) by an unknown mechanism (Barter et al., 2007b; Nissen et al., 2007) that likely reflected an off-target effect, as hypertension had not been observed in genetic CETP deficiency (Zhong et al., 1996) and some other classes of CETP inhibitors do not cause hypertension (Krishna et al., 2007; Kuivenhoven et al., 2005). Thus, it was not clear whether the clinical failure of torcetrapib was due to off-target toxicity or to a detrimental mechanism of action (Rader, 2007a; Tall, 2007; Tall et al., 2007). There has been considerable speculation that the strategy of CETP inhibition might lead to formation of HDL that is dysfunctional in terms of its ability to promote cholesterol efflux from macrophage foam cells (Brewer, 2004; Ishigami et al., 1994), that there might be a block in reverse cholesterol transport (RCT) from peripheral tissues to liver and stool (Rader, 2007a), or that HDL might be proinflammatory or pro-oxidant (Rader, 2007a; Singh et al., 2007). Here we will review recent clinical, cellular, and transgenic mouse studies relevant to these questions. These issues are also relevant to other approaches to raising HDL since exercise, alcohol, statins, bile acid sequestrants, and niacin all raise HDL at least in part by decreasing CETP levels or in vivo activity (NHLBI, 1984; de Haan et al., 2008; Fumeron et al., 1995; Morton and Greene, 1997; Schaefer and Asztalos, 2006; Seip et al., 1993). For example, HDL elevation in patients taking statins or bile acid sequestrants may signal a lower level of CETP-mediated transfer of HDL cholesteryl ester (CE) into very low-density lipoprotein (VLDL) remnants that have been cleared by upregulated hepatic LDL receptors.

A Recent Analysis of Clinical Data Identifies an Off-Target Toxicity of Torcetrapib, While Coronary Imaging Data Suggest Benefits of High HDL

Two recent reports have helped to elucidate whether the adverse clinical outcome in ILLUMINATE was related to the molecule

(torcetrapib) or the mechanism (CETP inhibition) (Barter et al., 2007b; Nissen et al., 2007). The first report documents an increase in mortality and morbidity from cardiovascular and noncardiovascular causes in patients receiving torcetrapib and atorvastatin (T+A) compared to atorvastatin (A) alone (Barter et al., 2007b). Torcetrapib therapy was associated with a significant worsening of the primary cardiovascular endpoint (the time to the first major cardiovascular event, including myocardial infarction, unstable angina, revascularization, and heart failure) as well as in total deaths, including an apparent excess of deaths from cancer and infectious disease. The excess of deaths from infectious disease and cancer in the group receiving torcetrapib was not paralleled by a similar increase in infections or cancers reported as adverse events, raising the possibility that these deaths resulted from torcetrapib-related cardiovascular toxicity as a terminal event complicating cancer or infection. However, HDL is known to modulate acute-phase responses, and it is conceivable that very high HDL in subjects treated with CETP inhibitors could dampen the inflammatory response to a severe infection (Rader, 2007a).

This study also revealed that patients receiving torcetrapib had significant reductions in serum potassium levels and increases in bicarbonate and in serum aldosterone levels (Barter et al., 2007b). While blood pressure changes were not predictive of clinical outcome, there was an excess of deaths and cardiovascular events in patients whose decreases in serum potassium or increases in serum bicarbonate levels were more than the median. In contrast, in patients who experienced the largest increases in HDL (i.e., above the median), there appeared to be a modest decrease in the primary cardiovascular endpoint. Aldosterone has a wide spectrum of vascular toxicities, including increased atherosclerosis (Keidar et al., 2004). Even though the difference in serum potassium levels between the T+A and A groups was small (0.1 mM), the levels were similar in magnitude to those seen in clinical trials of aldosterone antagonists for heart failure after myocardial infarction, in which the slight increases in serum potassium levels were associated with a beneficial clinical outcome (Pitt et al., 2003). The mechanisms of hyperaldosteronism are uncertain, but increases in aldosterone have been seen in torcetrapib-treated rats that naturally lack CETP. These studies strongly suggest significant off-target toxicity of torcetrapib related to hyperaldosteronism and hypokalemia. They also appear to be inconsistent with the hypothesis that direct toxic effects of HDL were responsible for the adverse cardiovascular outcome. However, unknown mechanism-related adverse effects of CETP inhibition on cardiovascular events could still be involved in the negative results of this study (Barter et al., 2007b).

In addition to the ILLUMINATE study, parallel imaging studies of coronaries and carotids showed no difference in the primary measures of atherosclerosis. However, there was a significant decrease in a secondary endpoint, measurement of coronary atherosclerosis volume (Nissen et al., 2007). Interestingly, a recent post hoc analysis showed that in the T+A group, there was a significant inverse relationship between the change in HDL cholesterol level and the primary measure of atherosclerosis, i.e., the percent atheroma volume (Nicholls et al., 2007). Indeed, there was significant regression of atherosclerosis in patients with the largest increases in HDL levels. There was no relationship between change in HDL and percent atheroma volume in the A group, suggesting that the effect was specific to torcetrapib treatment. Stratification of the data by change in serum potassium level revealed a substantial beneficial effect on percent atheroma volume in patients with the largest increases in HDL levels whose change in potassium was above the median. The intravascular ultrasound results appear to parallel the clinical outcome in ILLUMINATE and provide a strong suggestion that maximal HDL increases mediated by CETP inhibition reduce the volume of coronary atherosclerosis. However, these suggestions based on post hoc analyses (Barter et al., 2007b; Nissen et al., 2007; Nicholls et al., 2007) are only useful for generating hypotheses. They indicate that it may be worthwhile to evaluate other classes of CETP inhibitors that do not cause hyperaldosteronism or hypertension in randomized clinical trials. They also suggest that the maximal benefit of such HDL-raising therapies may only be attained at very high HDL cholesterol levels > 60 mg/dl. Interestingly, this parallels earlier observations on the relationship between genetic CETP deficiency and CHD, where a beneficial association was only apparent in subjects with HDL cholesterol > 60 mg/dl (Zhong et al., 1996). Similarly, CETP single-nucleotide polymorphisms that are associated with slightly higher HDL levels in the general population are not associated with significant alterations in CHD risk (Willer et al., 2008).

The Role of HDL, ABCA1, and ABCG1 in Macrophage Cholesterol Efflux

The ability of HDL and its major apolipoprotein, apoA-I, to stimulate efflux of cholesterol from macrophage foam cells in atheromatous blood vessels is thought to be central to its antiatherogenic mechanism and to represent the first step in an overall process of RCT (Figure 1). The ATP-binding cassette (ABC) transporter ABCA1, the mutant molecule in Tangier disease (Bodzioch et al., 1999; Brooks-Wilson et al., 1999; Rust et al., 1999), promotes net cholesterol efflux to lipid-poor apoA-I, while ABCG1 facilitates net cholesterol efflux to HDL particles (Kennedy et al., 2005; Wang et al., 2004). Deficiency of CETP results in a prominent increase in larger HDL-2 particles, and in complete CETP deficiency, there is a marked increase in the apoE-HDL fraction. HDL isolated from subjects with homozygous CETP deficiency showed enhanced ability to promote cholesterol efflux from macrophage foam cells. Depletion of apoE from the HDL fraction decreased its cholesterol efflux potential, and knockdown of macrophage ABCG1 also substantially reduced cholesterol efflux to HDL from CETP-deficient subjects (Matsuura et al., 2006). Mahley, Weisgraber, and coworkers (Mahley et al., 2006; Peters-Libeu et al., 2006) have shown that in reconstituted HDL particles, apoE resides in a superficial location among the phosphorylcholine head groups of phospholipids in contrast to more deeply embedded apoA-I molecules and have speculated that this may endow flexibility in the surface of HDL, permitting expansion of the core of HDL with CE as the lecithin:cholesterol acyltransferase (LCAT) reaction proceeds (Mahley et al., 2006). Importantly, a study of the cholesterol efflux potential of HDL isolated from subjects treated with torcetrapib showed moderately increased mean cholesterol efflux from macrophages using HDL from patients treated with the 60 mg dose (the dose used in phase 3 torcetrapib clinical studies), primarily due to an increase in HDL concentration, while

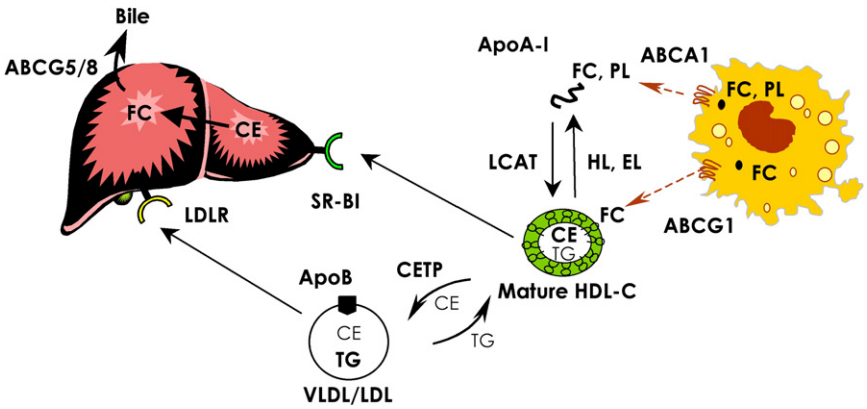

Figure 1. Role of HDL in Macrophage Cholesterol Efflux and Reverse Cholesterol Transport

Lipid-free or lipid-poor apolipoprotein A-I (apoA-I) can interact with the ATP-binding cassette (ABC) transporter A1 (ABCA1) in macrophage foam cells in atheroma, promoting efflux of free cholesterol (FC) and phospholipids (PL). This results in the formation of nascent high-density lipoprotein (HDL) particles that are further modified by lecithin:cholesterol acyltransferase (LCAT), generating cholesteryl esters (CE) and forming mature HDL. HDL particles are also formed by ABCA1 in the liver and intestine (not shown). Mature HDL particles can serve as acceptors for ABCG1-mediated cholesterol efflux; there is also cholesterol efflux to mature HDL by passive efflux (Yancey et al., 2003) and possibly other transporters as well. In humans and some other species, CE in HDL can be transferred to apoB-containing lipoproteins by cholesteryl ester transfer protein (CETP) and remnant lipoproteins subsequently cleared by low-density lipoprotein (LDL) receptors in the liver. Finally, HDL cholesterol can be taken up by the liver and subsequently secreted into the bile in a process that may involve selective lipid uptake by scavenger receptor BI (SR-BI). SR-BI is important in mice and rats, but its significance in humans is unknown.

HDL from subjects treated with a higher dose of 120 mg showed substantially greater cholesterol efflux potential due to both an increase in HDL concentration and an increased cholesterol efflux potential per unit mass HDL. More subjects showed an increase in apoE and LCAT in the HDL-2 at the higher dose, and the greater the increase in HDL level, the more the enrichment with apoE and LCAT (Yvan-Charvet et al., 2007a). These findings suggest that, at least in terms of cholesterol efflux potential monitored in these assays, HDL has normal or enhanced functionality (Matsuura et al., 2006; Yvan-Charvet et al., 2007a), paralleling the observation that subjects with larger HDL increases on torcetrapib showed more pronounced regression of coronary atherosclerosis (Nicholls et al., 2007).

A limitation of cell culture studies is that they are a static system. In vivo, the process of CE-triglyceride exchange mediated by CETP may be important in regenerating lipid-poor apoA-I to serve as substrate for ABCA1, and thus CETP inhibition could potentially have an adverse effect on ABCA1-mediated cholesterol efflux from foam cells (Wang et al., 2001). However, plasma apoA-I levels are increased in CETP deficiency, and the concentration of the pre-beta HDL fraction that may in part represent lipid-poor apoA-I is preserved or increased (Asztalos et al., 2004; Duverger et al., 1995; Inazu et al., 1990). Interestingly, patients treated with torcetrapib who had larger increases in apoA-I levels appeared to have better cardiovascular outcomes (Barter et al., 2007b). CETP deficiency probably increases plasma apoA-I levels by increasing HDL size and delaying catabolism of apoA-I (e.g., by decreasing renal filtration) (Ikewaki et al., 1993). There may be sufficient local remodeling of HDL in the arterial wall by lipases and phospholipid transfer protein (PLTP) secreted by macrophages to regenerate lipid-poor apoA-I (Tobias and Curtiss, 2005). This could lead to enhanced activities of both ABCA1 and ABCG1 lipid efflux pathways from foam cells in atheroma (Figure 2). Thus, CETP inhibition may enhance cholesterol efflux from macrophage foam cells via ABCG1, passive efflux, and ABCA1 pathways, leading to a regression of coronary atheroma at high levels of HDL.

CETP and Reverse Cholesterol Transport

While these studies highlight the likely beneficial effects of CETP deficiency on cholesterol efflux from foam cells, CETP inhibition in rabbits and humans does not stimulate the overall process of RCT (Brousseau et al., 2005). In fact, a recent study of macrophage RCT (the rate of movement of [^{3}H]cholesterol from macrophages injected into the peritoneal cavity into serum, liver, and feces) showed that adeno-associated virus (AAV)-CETP increased overall RCT in mice as measured by the movement of [^{3}H]cholesterol from macrophages into feces. The initial steps of [^{3}H]cholesterol movement from macrophages to serum were not significantly changed by CETP expression. This apparent stimulation of RCT into stool by AAV-CETP was not observed in *Ldlr*$^{-/-}$ mice, suggesting that CETP enhances RCT by promoting the movement of CE from HDL into apoB-containing lipoproteins with subsequent removal of remnants by LDLR (Tanigawa et al., 2007). One implication of these studies could be that in subjects treated with high doses of statins, upregulation of LDLR might play a pivotal role in CETP-mediated RCT, and thus the combination of CETP inhibitors and statins might have an adverse effect on RCT (Barter et al., 2007b; Nissen et al., 2007).

It is important to note, however, that the macrophage RCT method does not measure net fluxes of sterol, and movements of radioactivity may reflect isotope exchange processes that are responding to changes in sterol pool sizes in plasma or liver. For example, CETP expression appreciably lowered plasma cholesterol levels in *Ldlr*$^{+/+}$ mice, but not in *Ldlr*$^{-/-}$ mice (Tanigawa et al., 2007). Thus, the specific activity of macrophage-derived [^{3}H]cholesterol in plasma is higher in CETP transgenic mice versus nontransgenic mice on a *Ldlr*$^{+/+}$ background, but not in *Ldlr*$^{-/-}$ mice. The ability of CETP to increase movement of [^{3}H]cholesterol radioactivity from plasma into stool in the *Ldlr*$^{+/+}$ group might arise from the higher specific activity without indicating an increase in net flux of plasma sterol into feces in the group expressing CETP. Net sterol outputs were not reported in the Tanigawa et al. (2007) study. Moreover, the Tanigawa et al. (2007) studies in mice contrast with the earlier findings that fecal neutral sterol and bile acid excretion were not appreciably changed in humans treated with high doses of torcetrapib, whether or not the subjects received concomitant treatment with atorvastatin (Brousseau et al., 2005). In summary, it is likely that in the steady state, CETP inhibition does not change the overall rate of flux of cholesterol from peripheral tissues to liver

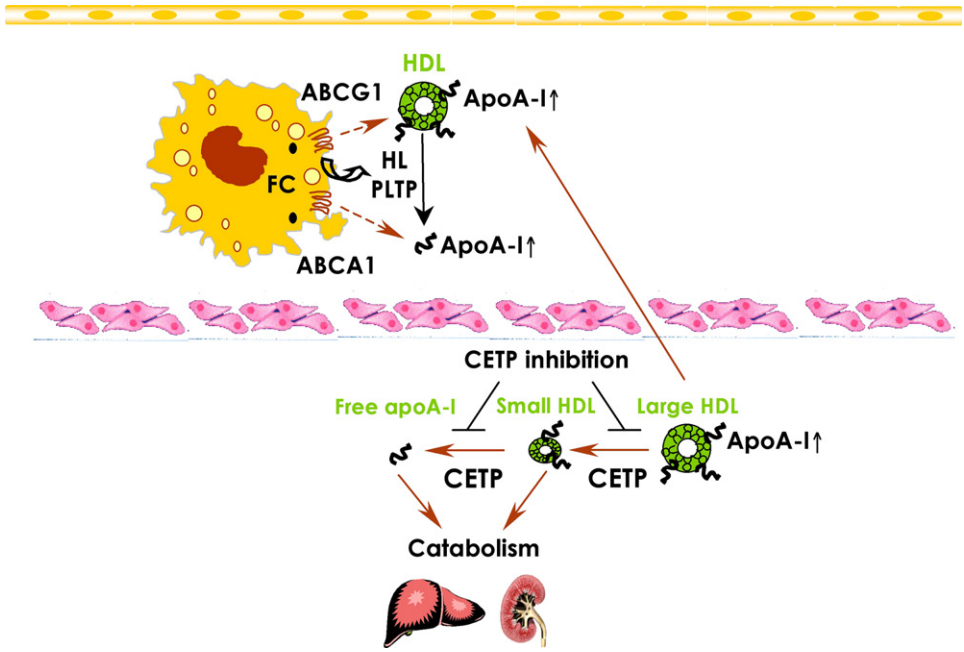

Figure 2. Hypothetical Scheme Showing How CETP Inhibition Could Lead to Improvements of Macrophage Cholesterol Efflux Involving Both ABCA1 and ABCG1 Transporters

Inhibition or genetic deficiency of CETP leads to accumulation of larger HDL in the plasma compartment due to impaired clearance of particles in the kidney and liver (Ikewaki et al., 1993). The reduction in catabolism of HDL-associated apoA-I leads to an increased pool of apoA-I and entry of increased numbers of large HDL particles into the artery wall. On entering the subendothelial space, large particles can act as acceptors for ABCG1-mediated cholesterol efflux, and there may also be sufficient local remodeling of HDL in the arterial wall by lipases (HL) and phospholipid transfer protein (PLTP) secreted by macrophages (Curtiss et al., 2006) to regenerate lipid-poor apoA-I, which in turn could also enhance the ABCA1 lipid efflux pathway from macrophage foam cells in atheroma. In addition, large HDL may promote efflux of cholesterol and oxysterols from endothelial cells, which express abundant ABCG1 (Hassan et al., 2006).

or stool; however, the pathway of cholesterol transport is altered, favoring transport in HDL and direct uptake in the liver rather than the indirect pathway of RCT involving transfer to the liver via VLDL/LDL (Figure 1). In this regard, the high-affinity binding of apoE-rich HDL to LDLR in CETP deficiency (Yamashita et al., 1990) and scavenger receptor BI (SR-BI)-mediated selective uptake of HDL CE (Trigatti et al., 2003) could play significant roles. In addition, the CE content of VLDL and intermediate-density lipoprotein (IDL) (remnant) particles is reduced, and the level of LDL is decreased due to increased hepatic uptake and possibly decreased conversion of VLDL to LDL (Ikewaki et al., 1995). Thus, any potential benefit of CETP inhibition derives not from an overall increase in RCT but rather from an altered lipoprotein profile with increased HDL and reduced levels of cholesterol in remnants and LDL, with consequent favorable interactions with cells in the arterial wall.

The Role of Different Cholesterol Efflux Pathways in Atherosclerosis

Several different potential cholesterol efflux pathways from macrophages to HDL have been described: passive or diffusional efflux, efflux associated with macrophage apoE secretion, SR-BI-mediated cholesterol efflux, and active cholesterol efflux mediated by the ABC transporters ABCA1 and ABCG1 (Tall, 2003; Yancey et al., 2003). Recent studies from several different laboratories indicate that together, ABCA1 and ABCG1 have the major role in mediating net cholesterol efflux from macrophages to HDL or serum (Adorni et al., 2007; Out et al., 2008b; Wang et al., 2007; Yvan-Charvet et al., 2007b, 2008). When one transporter is deficient, the other is induced as a result of sterol accumulation and liver X receptor (LXR) activation, resulting in mutual compensation in the activities of the two transporters (Ranalletta et al., 2006; Yvan-Charvet et al., 2007a). Macrophages with combined deficiency of ABCA1 and ABCG1 have major defects in cholesterol efflux to apoA-I, HDL, and serum, as well as in apoE secretion (Out et al., 2008b; Yvan-Charvet et al., 2007b). In one study, combined deficiency of ABCA1 and ABCG1 resulted in a 60% decrease in macrophage net cholesterol efflux to HDL (Yvan-Charvet et al., 2007b), while in another study, the decrease in cholesterol efflux was reported as 100% (Out et al., 2008b). A third set of studies suggested major roles of ABCA1 and ABCG1 in cholesterol-loaded macrophages and a relatively larger role of passive cholesterol efflux from cells to HDL, especially when macrophages are not loaded with cholesterol (Adorni et al., 2007). Compared to wild-type mice or mice with single deficiencies of ABCA1 or ABCG1, mice with combined deficiency of ABCA1 and ABCG1 showed much greater accumulation of cholesterol in the form of CE in peritoneal macrophages on either chow or high-cholesterol diets, as well as foam cell accumulation in various organs such as the spleen (Out et al., 2008a, 2008b; Yvan-Charvet et al., 2007b). Moreover, parallel studies of macrophage RCT showed major and additive effects of macrophage deficiency of ABCA1 and ABCG1 on movement of radiotracer into serum, liver, and feces (Wang et al., 2007). Since ABCA1 and ABCG1 mediate unidirectional cholesterol efflux from cells to HDL and HDL (Wang et al.,

2000, 2004, 2007; T.P. and A.R.T., unpublished data), these studies provide a minimum estimate of the contribution of ABCA1 and ABCG1 to macrophage cholesterol efflux to serum in vivo. Together, these recent studies have confirmed that ABCA1 and ABCG1 account for the major portion of the net cholesterol efflux from cholesterol-loaded macrophages to plasma lipoproteins in vivo.

The atherosclerosis studies provide a close parallel with the cholesterol efflux data and indicate that combined deficiency of ABCA1 and ABCG1 in bone marrow-derived cells results in a dramatic enhancement of atherosclerosis. Transplantation of *Abca1*$^{-/-}$ bone marrow into *Ldlr*$^{-/-}$ or *Apoe*$^{-/-}$ mice results in a modest increase in atherosclerosis (Aiello et al., 2002; van Eck et al., 2002). In contrast, transplantation of *Abcg1*$^{-/-}$ bone marrow into *Ldlr*$^{-/-}$ or *Apoe*$^{-/-}$ mice resulted in either no change or a decrease in atherosclerosis (Baldan et al., 2006; Out et al., 2008a; Ranalletta et al., 2006). The decrease in atherosclerosis was attributed to either compensatory upregulation of ABCA1 in *Abcg1*$^{-/-}$ macrophages (Ranalletta et al., 2006) or enhanced apoptosis of *Abcg1*$^{-/-}$ macrophages (Baldan et al., 2006). Importantly, transplantation of *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow into *Ldlr*$^{+/-}$ mice followed by feeding a high-cholesterol diet (1.25% cholesterol and 1% cholic acid) led to markedly increased atherosclerosis compared to mice receiving bone marrow with single knockout (KO) of ABCA1 or ABCG1 (Yvan-Charvet et al., 2007b). In addition, there was prominent accumulation of foam cells in the heart, spleen, and small intestine (Yvan-Charvet et al., 2007b). A similar study (Out et al., 2008a) involved transplantation of *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ bone marrow into *Ldlr*$^{-/-}$ mice fed a Western-type diet (0.15% cholesterol and saturated fat). This study also documented an increase in foam cells in various organs including spleen, small intestine, Peyer's patches, and liver. However, in contrast to the aforementioned study (Yvan-Charvet et al., 2007b) wherein plasma VLDL and LDL levels were similar in mice receiving wild-type or double-KO bone marrow, plasma VLDL and LDL levels in this study (Out et al., 2008a) were markedly decreased in the double-KO recipients, with differences between the studies probably reflecting the different cholesterol content of the diets. Nonetheless, atherosclerosis was increased in the double-KO recipients relative to the level of plasma cholesterol (Out et al., 2008a).

Cellular Mechanisms of Cholesterol Efflux via ABCA1 and ABCG1

ABCA1 promotes efflux of phospholipids and cholesterol to lipid-poor apoA-I in a process that involves the direct binding of apoA-I to the transporter (Oram et al., 2000; Wang et al., 2000). Most likely ABCA1 translocates phospholipids from the inner to the outer membrane leaflet of the plasma membrane, perhaps creating outward curvature and packing defects in the membrane; this may allow interpolation of amphipathic helices of apoA-I into the membrane and formation of nascent HDL particles (Vedhachalam et al., 2007). Cholesterol and phospholipid efflux via ABCA1 appear to occur simultaneously (Smith et al., 2004), and it is possible that the transporter also translocates cholesterol onto the forming HDL particle (Gillotte-Taylor et al., 2002). In contrast, ABCG1 promotes efflux of cholesterol onto a variety of lipoprotein particles, including HDL, LDL, phospholipid vesicles, and cyclodextrin, but does not appear to bind lipoprotein particles (Wang et al., 2004, 2006). Overexpression of ABCG1 also promotes efflux of choline-containing phospholipids onto HDL (Wang et al., 2004), likely including both sphingomyelin and phosphatidylcholine (Kobayashi et al., 2006). Following activation of LXRs in macrophages, the ability of ABCG1 to promote cholesterol efflux correlates with the appearance of ABCG1 in the plasma membrane (Wang et al., 2006).

Two potential mechanisms of sterol efflux by ABCG transporters have been proposed (Figure 3):

(1) Small (2003) has suggested that ABCG5 and ABCG8, heterodimeric transporters that promote secretion of cholesterol and plant sterols into bile, mediate protrusion of the hydrophobic sterol molecule into the aqueous phase, followed by collision with a micelle. By analogy, ABCG1 could promote protrusion of cholesterol from the plasma membrane followed by transient collision with an HDL particle (Figure 3A). Since there is very little energetic barrier to the movement of cholesterol between the two leaflets of a bilayer membrane (Small, 2003), it seems unlikely that ABCG1 simply translocates cholesterol from the inner to the outer plasma membrane.

(2) Alternatively, ABCG1 could change the organization of phospholipids in the plasma membrane such that the membrane more readily releases sterol and phospholipids to lipoprotein acceptors (Figure 3B). This model is consistent with the nonspecific nature and lack of binding of lipoprotein acceptors by ABCG1.

It is notable that ABCG1 appears to promote movement of cholesterol from the ER to the plasma membrane, manifested as decreased cellular ACAT activity and decreased activity of SREBP-2 target genes, even in the absence of extracellular lipoprotein acceptors (Wang et al., 2006). Thus, ABCG1 appears to increase the affinity of the plasma membrane for ER-derived sterols even while releasing sterols more readily to extracellular lipoprotein acceptors (Wang et al., 2006), perhaps secondary to translocation and efflux of membrane phospholipids.

A Potential Role of HDL and ABCG1 in Macrophage Apoptosis and Inflammation in Plaques

Ruptured atherosclerotic plaques are characterized by thin caps, large necrotic cores, and increased numbers of apoptotic macrophages and smooth muscle cells. HDL was found to protect macrophages from apoptosis induced by oxidized LDL, or by loading with free cholesterol (Cui et al., 2007; Terasaka et al., 2007). In the case of oxidized LDL-induced apoptosis, the protective effect of HDL was abolished in *Abcg1*$^{-/-}$ macrophages (Terasaka et al., 2007). In contrast, for free cholesterol (FC)-induced apoptosis, knockout of both ABCG1 and ABCA1 was required to see an abolition of the protective effect of HDL (Yvan-Charvet et al., 2007a). ABCG1 has a specific role in promoting cellular efflux of sterols modified at the 7 position such as 7-ketocholesterol (7-KC; Terasaka et al., 2007). 7-KC is a spontaneously formed cholesterol oxidation product that is present in processed foods and high-cholesterol diets and is the most abundant oxysterol in oxidized LDL and in human atherosclerotic plaques (Brown et al., 1996; Brown and Jessup, 1999; Vine et al., 1998). Dietary 7-KC is normally absorbed on

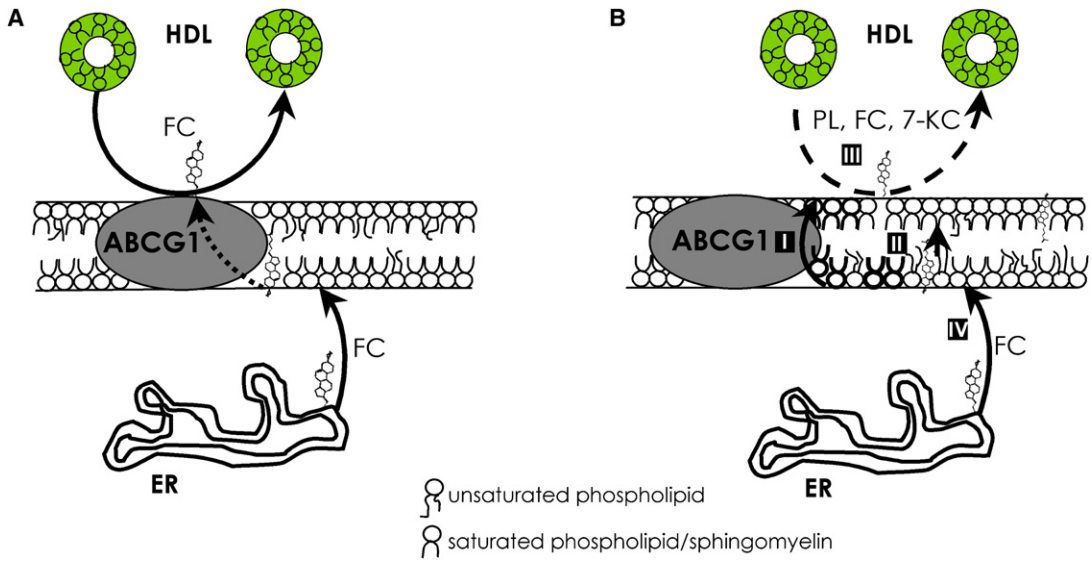

Figure 3. Two Proposed Mechanisms of ABCG1-Mediated Sterol Efflux

(A) One model suggests that ABCG transporters accomplish FC transfer by helping sterol molecules to overcome the energy barrier for entry into the hydrophilic water layer perhaps by utilizing ATP to promote protrusion of the cholesterol molecule into water, followed by a transient collision with an acceptor (Small, 2003), in this case HDL.

(B) In the second model, the ability of ABCG1 to promote PL efflux (Kobayashi et al., 2006) points to a function as a phospholipid flippase mediating the transfer of PL from the inner to the outer leaflet (I). This could lead to such an extensive change in the equilibrium of membrane components that the outer leaflet becomes more attractive to sterol (e.g., an increased content of sphingomyelin or saturated phosphatidylcholine), followed by transbilayer diffusion of cholesterol molecules toward the outer leaflet (II), where they can dissociate onto HDL particles perhaps following nonspecific binding of HDL to the plasma membrane (III) (Tabas and Tall, 1984).

In both models, the movement of cholesterol from the inner to the outer membrane is followed by carrier-facilitated diffusion from cellular organelles, notably the endoplasmic reticulum (ER; IV), leading to altered sterol-mediated ER regulation of cholesterol homeostasis (Maxfield and Menon, 2006; Wang et al., 2006).

chylomicrons, rapidly cleared from the circulation in remnants, and converted into bile salts in the liver. However, 7-KC is apparently cytotoxic at some concentrations found in vivo, inducing apoptosis and necrosis of endothelial cells and macrophages. HDL and ABCG1 have a specific role in promoting efflux of 7-KC and 7-β-OH cholesterol in transfected 293 cells and macrophages, while ABCA1 and apoA-I have no ability to stimulate efflux of these oxysterols (Terasaka et al., 2007). Other oxysterols such as 25-OH cholesterol can undergo efflux by both ABCA1 and ABCG1 pathways. Moreover, *Abcg1*$^{-/-}$ mice fed a Western diet showed prominent accumulation of 7-KC in macrophages (Terasaka et al., 2007). ABCG1 is also highly expressed in endothelial cells (O'Connell et al., 2004). The role of ABCG1 in promoting efflux of 7-KC suggests that large HDL-2 particles that promote sterol efflux via ABCG1 may have a particular role in protecting endothelial cells and macrophages from the deleterious effects of oxysterols consumed in the diet or formed on LDL (Terasaka et al., 2007). This could be important in maintaining normal endothelial functions and in atherosclerotic plaque stabilization (Libby et al., 2002).

In addition to the severe defect in cholesterol efflux and apoE secretion, *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages showed increased mRNA and secretion of chemokines and inflammatory cytokines (Yvan-Charvet et al., 2007b). Single KO of ABCG1 produced a similar, though milder, defect in secretion of inflammatory cytokines and chemokines, while KO of ABCA1 had minimal effects (Yvan-Charvet et al., 2007b). The HDL/ABCG1 pathway may have a specific role in decreasing macrophage inflammatory and chemokine responses (Yvan-Charvet et al., 2007b). Thus, HDL/ABCG1 may be important in protecting advanced plaques from macrophage apoptosis and inflammatory responses, suggesting a role in plaque stabilization and acute coronary syndromes.

Other potential anti-inflammatory properties of HDL may be related to its ability to take up oxidized phospholipids from other lipoproteins or cells and to its content of PAF acetylhydrolase (also known as lipoprotein-associated PLA-2), an enzyme that can break down short-chain oxidized phospholipids. In *Apoe*$^{-/-}$ mice, the ability of HDL to promote migration of dendritic cells out of the skin (Angeli et al., 2004), and perhaps by inference from arteries, may depend on its content of PAF acetylhydrolase (Karasawa, 2006). While the antioxidant and anti-inflammatory properties of HDL may be important in its antiatherogenic effects, oxidative and inflammatory processes may themselves impair the function of HDL. Thus, HDL isolated from subjects with coronary artery disease may lose its anti-inflammatory properties (Vaisar et al., 2007). Modification of apoA-I by macrophage-derived myeloperoxidase can lead to chlorination of apoA-I on specific amino acid residues and an impairment of cholesterol efflux by ABCA1 (Shao et al., 2005, 2006; Zheng et al., 2004, 2005).

Multiple Steps in the Pathogenesis of Atherosclerosis May Be Beneficially Affected by HDL

Atherosclerosis is initiated by the retention of apoB-containing lipoproteins on proteoglycans in the arterial matrix in thickened areas of the arterial intima (Gustafsson and Boren, 2004; Tabas et al., 2007). Subsequent modification of LDL by aggregation

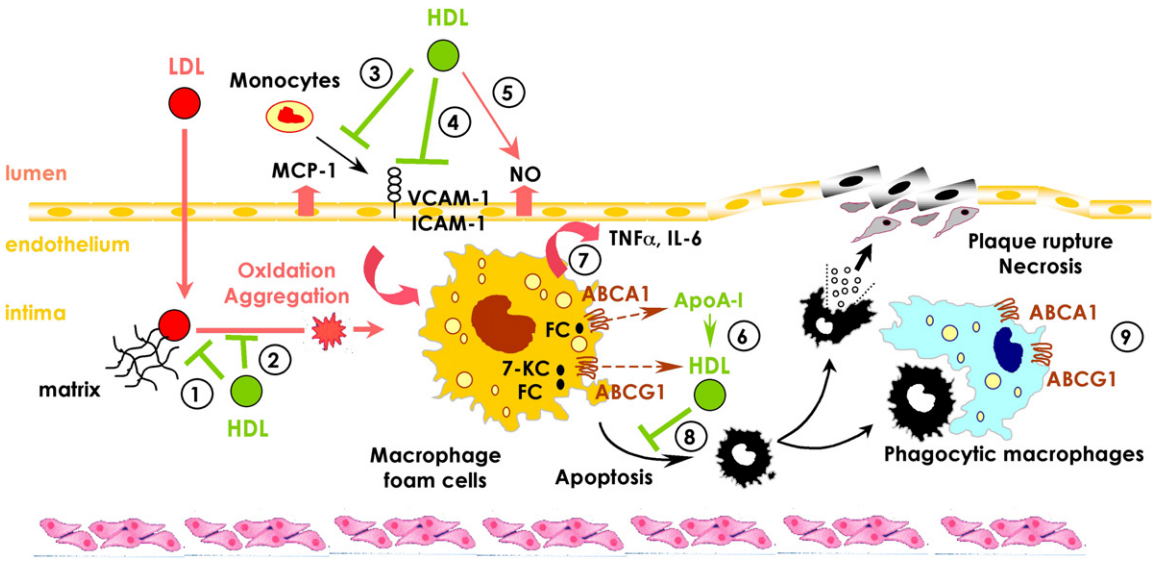

Figure 4. Potential Protective Effects of HDL on the Different Steps of Atherogenesis and Plaque Disruption

HDL can compete with the proteoglycan-rich matrix for binding of LDL particles limiting their retention (1) and can inhibit their modification by aggregation or oxidation, preventing a cascade of events that comprise the macrophage-dominated inflammatory process that triggers the cascade of events leading to atherosclerosis (2). HDL inhibits the attraction of monocytes to the endothelium by downregulating the expression of the chemoattractant cytokine MCP-1 (3). The binding of monocytes to the endothelium can also be inhibited by HDL by downregulation of the expression of the cell adhesion molecules VCAM-1 and ICAM-1 (4) and upregulation of endothelial cell nitric oxide (NO) release (5). HDL promotes macrophage cholesterol efflux via ABCA1 and ABCG1 (6) and thus reduces macrophage expression of inflammatory cytokines (7) as well as macrophage apoptosis induced by loading with unesterified cholesterol or oxidized LDL (8). Finally, HDL may help to preserve the viability of phagocytic macrophages by promoting massive cholesterol efflux after ingestion of cholesterol-enriched apoptotic cells (9), preventing postapoptotic necrosis with consequent inflammatory effects that contribute to plaque rupture.

or oxidation leads to a cascade of events that comprise the macrophage-dominated modified inflammatory response of atherosclerosis. Almost every step in this process has been reported to be favorably influenced by HDL. apoE-containing HDL can compete with the proteoglycan-rich matrix for binding of LDL (Figure 4, step 1) (Saxena et al., 1993), and HDL and apoA-I can inhibit LDL aggregation (Khoo et al., 1990). Moreover, HDL and its major apolipoprotein apoA-I have a variety of antioxidant properties that can reduce oxidation of LDL (step 2) (Navab et al., 2000a, 2000b; Watson et al., 1995). Oxidized phospholipids, hypercholesterolemia, and disturbed blood flow induce activation of endothelial cell NF-κB, resulting in upregulation of MCP-1, VCAM-1, and ICAM-1, causing attraction and binding of monocytes to the endothelium (Libby, 2002). HDL can inhibit the binding of monocytes to the endothelium by downregulating expression of cell adhesion molecules (Cockerill et al., 1995) (step 4). A variety of studies indicate that HDL enhances endothelial cell nitric oxide (NO) release (Assanasen et al., 2005; Gong et al., 2003; Nofer et al., 2004; Yuhanna et al., 2001), and NO can inhibit the binding of monocytes to the endothelium (step 5). LDL modified by aggregation or oxidation can be taken up by macrophages, inducing foam cell formation. HDL promotes macrophage cholesterol efflux via ABCA1 and ABCG1, and probably other pathways as well (step 6). As noted above, HDL and apoA-I acting via ABCA1 and ABCG1 likely reduce macrophage expression of inflammatory genes and chemokines (step 7). ABCG1 is highly expressed in vascular endothelium, and this could be relevant to the mechanisms by which HDL can reduce expression of cell adhesion molecules (Hassan et al., 2006; O'Connell et al., 2004). The activity of these two transporters also reduces apoptosis of macrophages induced by loading with unesterified cholesterol or oxidized LDL (step 8) (Terasaka et al., 2007). Finally, the clearance of cholesterol-loaded apoptotic cells by phagocytic macrophages is important in preventing postapopotic necrosis and inflammation (Tabas, 2005). Macrophages that have phagocytosed cholesterol-enriched apoptotic cells show marked induction of ABCA1 and ABCG1 expression (step 9) and massive cholesterol efflux, which helps to preserve their viability, likely preventing oxysterol- and FC-induced toxicity in phagocytes (Cui et al., 2007; Gerbod-Giannone et al., 2006).

Conclusion and Perspective

The failure of the ILLUMINATE study represents a watershed in the development of HDL-directed therapies. Different observers have had very different reactions to this study and its adverse outcome. On the one hand, the failure could signal the end of the quest for HDL-directed therapies and lead to refocusing on newer mechanisms of VLDL/LDL lowering (Cohen et al., 2006; Cuchel et al., 2007; Tall, 2006) or vessel wall targets. On the other hand, the post hoc analyses of the torcetrapib studies strongly suggest that sufficient increases in large HDL-2 particles may lead to a regression of coronary atherosclerosis and have identified a plausible mechanism of off-target toxicity of torcetrapib. These findings suggest that HDL increases due to CETP inhibitors, and perhaps by analogy niacin, may result in functional HDL. In parallel, basic research has revealed the importance of the ABCG1 pathway in mediating cellular sterol efflux to such large HDL particles. Treatments that increase cholesterol efflux via ABCA1 and/or ABCG1 are likely to be beneficial for atherosclerosis, though perhaps with a different spectrum of clinical

effects. Drugs that increase expression of apoA-I in the liver, or infusion of apoA-I or peptides derived from apoA-I that have been optimized for interaction with ABCA1 or LCAT, are also likely to be beneficial (Belalcazar et al., 2003; Linsel-Nitschke and Tall, 2005; Navab et al., 2006; Nissen et al., 2003; Rader, 2007b). These approaches may also lead to increased formation and levels of HDL particles that can in turn promote efflux of sterols via ABCG1. Therapeutic strategies that upregulate both ABCA1 and ABCG1 transporters may also be beneficial, as low levels of the transporters themselves may be rate limiting for cellular cholesterol efflux in some subjects with CHD (Trogan et al., 2006). Thus, assuming problems of increased fatty liver can be solved (Repa et al., 2000; Schultz et al., 2000), LXR activators might be ideal agents for increasing a variety of molecules involved in cholesterol efflux and transport (Bradley et al., 2007; Scott, 2007)—for example, restricting LXR activation to the small intestine might result in an increase in intestinal HDL formation via ABCA1, without producing fatty liver (Brunham et al., 2006).

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