Macrophages in the arterial intima take up modified LDL and become cholesterol-ester laden foam cells, which are the primary cell type in newly formed fatty streak lesions, and which play an important role throughout lesion progression and plaque vulnerability. Macrophages can unload their excess cellular cholesterol stores via lipid efflux, the first step in the protective reverse cholesterol transport pathway. In this pathway, efflux of cellular cholesterol to extracellular acceptors, such as HDL and lipid-poor apolipoproteins, targets this cholesterol for delivery to the liver for metabolism and direct excretion into the bile. The mechanisms of cellular cholesterol efflux are the focus of intensive research, and this field was advanced greatly by the discovery of ABCA1 as the Tangier disease gene in 1999. Tangier disease subjects have almost no plasma HDL, and their cells have a complete deficiency in cholesterol and phospholipid efflux to apolipoprotein A-I (apoAI) and a partial defect in lipid efflux to HDL. Thus, it was apparent that there is more than one pathway for lipid efflux to HDL. In 2001, Schmitz and colleagues demonstrated that ABCG1, another member of the ABC gene superfamily, was upregulated by cholesterol in human macrophages, and that an ABCG1 antisense oligonucleotide that reduced ABCG1 expression also decreased lipid efflux to HDL. The role of ABCG1 in lipid efflux was confirmed in 2004 when Wang and colleagues and Edwards and colleagues showed that ABCG1 transfected cells had increased cholesterol efflux to HDL.

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In the current issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Wang et al provide novel insights both into the regulation of ABCG1 activity and into aspects of the mechanism of ABCG1-mediated lipid efflux, which differentiates it from the mechanism of ABCA1-mediated lipid efflux. Their essential discovery is that treatment of cells with an LXR agonist, or cholesterol loading of cells that leads to endogenous activation of LXR, causes a redistribution of ABCG1 from intracellular organelles to the plasma membrane. This ABCG1 redistribution is associated with increased cholesterol efflux to HDL, as well as to cyclodextrin, a nonspecific acceptor of cell surface cholesterol. Wang et al found that LXR agonist treatment of mouse macrophages increased the mass of cholesterol released from cells to HDL. In contrast, LXR agonists did not increase cholesterol release to HDL from macrophages isolated from ABCG1-deficient mice. However, the basal cholesterol efflux to HDL was not greatly reduced comparing the ABCG1-deficient and wild-type macrophages, implying the existence of other efflux pathways to HDL. They also tested the effect of another related protein, ABCG4, which they had previously shown to mediate cholesterol efflux to HDL in transfected HEK293 cells. However, macrophages from ABCG4-deficient mice, compared with wild-type, had equivalent basal and LXR agonist–induced cholesterol efflux to HDL, implying that overexpression of ABCG4 in HEK293 cells did not correlate with the physiological effect of this protein in macrophages. The authors in this study used an appropriate cholesterol mass assay to measure efflux that avoids a pitfall of cholesterol radiolabeled cells, in which cholesterol exchange between the cells and HDL cannot be distinguished from net cholesterol efflux. In addition, they used HDL2, rather than total HDL, as the extracellular acceptor, and this subclass of HDL is not used efficiently by the ABCA1-mediated efflux pathway.

In the next series of studies, Wang et al used sucrose density gradients of cell lysates to demonstrate that LXR agonist treatment of mouse macrophages, as well as a human monocyte/macrophage cell line, led to movement of ABCG1 from intracellular organelles, principally the golgi, to the plasma membrane. This cellular redistribution of ABCG1 in the human cells was also observed after cholesterol loading, a condition which activates LXR endogenously. The LXR-mediated increase in ABCG1 on the plasma membrane was confirmed by cell surface biotinylation and intact cell protease susceptibility. However, the mechanism by which LXR activation led to ABCG1 redistribution is not yet characterized, and it may involve regulation of vesicular traffic or alternative ABCG1 mRNA splicing, as LXR agonists have been reported to shift the splice variant profile observed in mouse tissues. Wang et al then demonstrated that LXR activation not only leads to increased efflux to HDL, but also to cyclodextrin, a nonspecific acceptor of cell surface free cholesterol (FC), which was partially blocked by siRNA mediated down regulation of ABCG1 levels. The LXR-induced increase in cholesterol efflux to cyclodextrin can be interpreted as an increase in cell surface FC. An ABCG1-mediated increase in cell surface FC has been previously observed by Oram’s laboratory. Finally, Wang et al implied that the endoplasmic reticulum (ER) pool of FC was increased in cholesterol-loaded macrophages on siRNA-mediated inhibition of ABCG1 expression both in the presence or absence of an LXR agonist. This was indirectly demonstrated by an increase in the rate of cholesterol esterification (which occurs in the ER) and a decrease in the levels of SREBP2 target genes (SREBP2 processing is dependent...
on low levels of FC in the ER). Stated the other way around, increased ABCG1 activity should lead to decreased FC in the ER, although this was not directly addressed in Wang’s article, nor were the AGGCI1 knockout macrophages used to confirm this point. The effect of ABCG1 on cholesterol ester formation was congruent with in vivo observations from Edwards’ laboratory, in which ABCG1 knockout macrophages and hepatocytes accumulated cholesterol esters; although, this could be solely attributable to decreased efflux rather than a direct effect on FC trafficking.10 Edwards’ laboratory also found that ABCG1 knockout hepatocytes had increased levels of SREBP2 target mRNAs,9 similar to the ABCG1 siRNA effect in macrophages reported here by Wang.6 In contrast, Oram’s laboratory reported that ABCG1 overexpression in BHK cells led to increased, instead of decreased, cholesterol ester formation,8 but this difference could be partially attributable the use of different cell types, as macrophages may have special mechanisms to handle the massive amounts of cholesterol that they can take up via scavenger receptors or phagocytosis.

So, how does this new data fit into the larger picture of pathways for cholesterol efflux? ABCG1, ABCA1, and scavenger receptor BI (SR-BI) make up the known macrophage-expressed proteins that play a direct role in cholesterol efflux. As far as the mechanism of efflux and the preferred acceptors, ABCA1 appears unique, whereas ABCG1 and SR-BI have some similarities. ABCA1 uses lipid-free and lipid-poor exchangeable apolipoproteins, such as apoAI or apoE, as its preferred acceptors. In fact, even recombinant apoAI-phospholipid (PL) discs are not efficient ABCA1-mediated cholesterol acceptors.10 The mechanism of lipid efflux by ABCA1 involves binding and uptake of the acceptor, followed by rescretion of the acceptor in a complex with PL and FC.7,11,12 Although Oram has observed an increase in cell surface FC in transfected cells that overexpress ABCA1, using susceptibility to cholesterol oxidase at 37°C,13 my own studies have found no evidence of an ABCA1-mediated increase in cell surface FC, using cycloexctrin extractable cholesterol at 20°C in a murine macrophage cell line.12 In fact, two separate labs using different methods have found that an intracellular pool of FC is the primary pool that is used in ABCA1-mediated cholesterol efflux.14,15 In contrast, both ABCG1 and SR-BI use HDL, and not lipid-free apolipoproteins, as cholesterol acceptors.4,6,8,16 Both ABCG1 and SR-BI increase the FC concentration on the plasma membrane, as evidenced by increased cholesterol oxidase susceptibility and increased FC efflux to cycloexctrin.4,6,8,16 ABCG1 expression does not lead to appreciable HDL binding, and although SR-BI does have HDL binding activity and it can mediate HDL uptake, the extent that HDL binding plays a role in SR-BI-mediated lipid efflux is not clear.

For all three of these membrane proteins that mediate lipid efflux, cellular trafficking plays an important role in their activity. Wang et al demonstrate in their current article that LXR-mediated induction of ABCG1 activity is accompanied by a shift from an intracellular to a plasma membrane pool accompanied by an increase in plasma membrane FC.6 Neufeld has elegantly shown that ABCA1 traffics bidirectionally between an intracellular vesicular pool and the plasma membrane.7 Therefore, Yokoyama’s and my labs have shown that different inhibitors of ABCA1 activity act by trapping ABCA1 on the plasma membrane leading to decreased turnover and an increase in total and cell surface ABCA1 levels, supporting the concept that ABCA1 endocytic recycling is required for its lipid efflux activity.18,19 SR-BI trafficking has been appreciated in hepatocyte mediated selective cholesterol ester uptake via endocytic recycling of HDL20; although the role of HDL uptake and recycling in this process remains controversial.21 SR-BI-mediated HDL uptake and rescretion have also been proposed to play a direct role in lipid efflux to HDL.22,23

A lot remains to be discovered about lipid efflux. We don’t know the identity of other cell surface proteins that may facilitate lipid efflux. We also don’t know the extent that simple aqueous diffusion, not mediated by cell surface protein, plays in cholesterol efflux to HDL. And, we really understand very little about the molecular mechanism of apoAI lipidation by ABCA1, and how ABCG1 and SR-BI facilitate the transfer cellular cholesterol onto HDL.

References


Insight Into ABCG1-Mediated Cholesterol Efflux
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