LXR-Induced Redistribution of ABCG1 to Plasma Membrane in Macrophages Enhances Cholesterol Mass Efflux to HDL

Nan Wang, Mollie Ranalletta, Fumihiko Matsuura, Felix Peng, Alan R. Tall

Objectives—This study examines the ABCG1-mediated cholesterol efflux and intracellular cholesterol transport by studying the ABCG1 localization and function in macrophages.

Methods and Results—HEK 293 cell overexpressing ABCG1, RNA interference, or macrophages from ABCG1 or ABCG4 knockout mice were used. ABCG1 but not ABCG4 had a major role in the increased cholesterol mass efflux produced by treatment of macrophages with LXR activators. In 293 cells, ABCG1 was found in the plasma membrane, Golgi, and recycling endosomes. In contrast, in basal macrophages, ABCG1 was predominantly intracellular, and redistributed to the plasma membrane after LXR activation. LXR activation increased macrophage cholesterol efflux to high-density lipoprotein (HDL), low-density lipoprotein (LDL), and cyclodextrin in an ABCG1-dependent fashion. Suppression of ABCG1 expression increased cholesteryl ester formation and decreased SREBP2 target gene expression in macrophages, even in the absence of HDL acceptors.

Conclusions—LXR activation induces redistribution of ABCG1 from intracellular sites to the plasma membrane and increases cholesterol mass efflux to HDL in an ABCG1-dependent fashion. ABCG1 acts in the macrophage plasma membrane to increase the availability of cholesterol to a variety of lipoprotein and nonlipoprotein acceptors while limiting the accumulation of cholesterol in the endoplasmic reticulum. (Arterioscler Thromb Vasc Biol. 2006;26:1310-1316.)

Key Words: ABCG1 ▪ cholesterol efflux ▪ high-density lipoprotein ▪ liver X receptor ▪ macrophage

Epidemiological studies have revealed an inverse correlation between plasma high-density lipoprotein (HDL) cholesterol levels and atherosclerosis1,2 and low HDL is the most common lipoprotein abnormality in men with coronary heart disease.3 Reverse cholesterol transport, ie, the transport of excess cholesterol from macrophage foam cells in the arterial wall to the liver, has been proposed as a major mechanism to explain for the protective effect of HDL.4 However, the molecular and cellular mechanisms responsible for the various steps of reverse cholesterol transport are not fully understood. Several different pathways have been implicated in cholesterol efflux from macrophage foam cells to HDL or its apolipoproteins.5,6 ABCA1 is a membrane transporter that binds apoA-1 and acts in the plasma membrane and endosomal system to promote cellular cholesterol efflux to lipid-poor apolipoproteins, such as apoA-1 and apoE.7,8 However, ABCA1 interacts poorly with HDL2 and HDL3 that constitute the bulk of plasma HDL.7 Recently, ABCG1 has been identified as a membrane transporter that promotes macrophage cholesterol efflux to HDL.9,10 Overexpression of ABCG1 increased cholesterol efflux to HDL but not lipid-poor apoA-1,7 whereas suppression of ABCG1 expression, by RNA interference or by targeted disruption of the ABCG1 gene, reduced the efflux of radioactive cholesterol from cholesterol-loaded macrophages to HDL.9,11 In macrophages, LXR activation promotes isotopic cholesterol efflux to HDL particles,9,11 and this induction is almost absent in ABCG1−/− macrophages.11 Together these studies suggested a major role of ABCG1 in promoting isotopic cholesterol efflux from macrophage foam cells to HDL. However, because isotopic efflux to HDL can arise either from net mass movement or from isotopic exchange, these studies do not disclose the role of the ABCG1 pathway in promoting mass cholesterol efflux to HDL.

In addition to ABCG1, we found that ABCG4, a close relative of ABCG1, could also promote cholesterol efflux to HDL when overexpressed in HEK293 cells.9 Knockdown of ABCG4 expression by siRNA suggested a possible lesser role of ABCG4 in cholesterol efflux from macrophages, but studies were inconclusive.9 In this study, we assessed the roles of ABCG1 and ABCG4 in cholesterol efflux to HDL from macrophages with or without LXR activation.

We previously reported that ABCG1 overexpression in 293 cells led to increased cholesterol efflux to a variety of
lipoprotein and nonlipoprotein acceptors, including HDL, low-density lipoprotein (LDL), and cyclodextrin, but did not increase cell association of HDL, suggesting that ABCG1 acts to increase availability of cholesterol in the plasma membrane.9 Consistent with this concept, Vaughan and Oram recently used biotinylation to show plasma membrane localization of an epitope-tagged ABCG1 in a baby hamster kidney (BHK) cell overexpression system, and demonstrated an increase in availability of cholesterol to cholesterol oxidase.12 However, the possible localization of ABCG1 in different intracellular pools and cellular localization of endogenous ABCG1 in macrophages or other cells have not been reported.

Materials and Methods

Reagents and Chemicals
Polyclonal anti-ABCG1 antibodies, H-65 (for immunofluorescence microscopy) and E-20 (for Western analysis) were from Santa Cruz Biotechnology (Santa Cruz, Calif).

Plasmid Constructs and Cell Transfection
The plasmid constructs expressing mouse ABCG1 and transfection were described previously.9 ABCG1-Flag or ABCG1-GFP expression plasmid was prepared by tagging the epitope at C-terminus of mouse ABCG1.

Cellular Lipid Efflux Assays
Cholesterol efflux assay using [3H]cholesterol labeled cells were performed as described before.9 For mass cholesterol efflux, macrophages were treated with or without the indicated siRNA for 48 hours in the presence or absence of 3 mol/L TO901317. During the last 16 hours of treatment, acetyl-LDL (50 μg/mL acetyl-LDL protein) was added. Cells were then washed and cholesterol mass efflux was initiated by adding the indicated amount of HDL2 and proceeded for 8 hours. The lipid fraction was extracted from the media or cell lysates with hexane:isopropanol (3:2, v:v) and β-sitosterol (5 μg/sample) was added as the internal standard. After drying under nitrogen gas, the mass of cholesterol dissolved in chloroform was determined using gas chromatography.

Small Interfering RNA (siRNA)-Mediated Macrophage RNA Interference
The ABCG1 target sequence selected for siRNA synthesis was 5′-TCGTATCTTATCTGAGAA-3′. Transfection of macrophages with siRNA and cholesterol efflux assay were performed as described previously.9 Levels of ABCG1 or ABCG4 mRNAs normalized against β-actin mRNA were determined using Taqman real-time quantitative reverse-transcriptase (RT) polymerase chain reaction (PCR).

Animals
ABCG1−/− and ABCG4−/− mice were derived from ABCG1+/− or ABCG4+/− mice obtained from Deltagen Inc. A stretch of 26 nucleotides encoding 9 amino acids in ATP-binding Walker A motif of ABCG4 was deleted in these knockout mice. RT-PCR using total RNA from the brain or macrophages of ABCG4−/− mice failed to detect ABCG4 mRNA, whereas a robust ABCG4 RT-PCR product was generated from the wild-type tissues (not shown).

Subcellular Fractionation
Peritoneal macrophages were cultured for 24 hours in 10% FBS and DMEM and then scraped and collected by centrifugation in ice cold phosphate buffered saline. The cells were processed and fractionated according to a procedure by Li et al.13 except that a continuous 0.58- to 1.1-mol/L sucrose density gradient centrifugation was used.

Limited Proteolysis
Macrophages were treated with indicated amount of trypsin in DMEM medium at 37°C or room temperature for the indicated period, washed 5 times with DMEM containing 0.2% bovine serum albumin, and lysed with lysis buffer containing a protease inhibitor cocktail and soybean trypsin inhibitor. The cell lysates were subjected to Western analysis.

Cell Surface Protein Biotinylation
Cell surface protein biotinylation, Western analysis, and quantification of the total cellular and cell surface ABCG1 were performed similarly as described previously.14

Results

Role of ABCG1 and ABCG4 in Mass Cholesterol Efflux From Macrophages
LXR activation significantly increased cholesterol mass efflux to HDL2 from wild-type macrophages (Figure 1A). This increased cholesterol mass efflux was markedly reduced in ABCG1-deficient macrophages. In contrast, the basal cholesterol mass efflux was only slightly decreased by ABCG1 deficiency (Figure 1A). These findings show the essential role of ABCG1 in promoting cholesterol mass efflux to HDL in LXR-activated macrophages but also indicate a residual component of mass efflux to HDL in the basal state that is not attributable to ABCG1.

We also examined the effect of acute suppression of ABCG1 expression on macrophage cholesterol mass efflux using a synthetic interfering RNA (siRNA) targeting mouse ABCG1 as described previously.9 On LXR activation by TO901317, ABCG1 protein levels were increased by ~4-fold and ABCG1 mRNA levels were increased by ~10-fold (not shown) and an siRNA selected previously to effectively downregulate ABCG1 mRNA levels reduced ABCG1 protein levels by ~70% (Figure 1B). Consistent with the results using ABCG1−/− macrophages, acute suppression of ABCG1 expression also reduced cholesterol mass efflux to HDL2 in LXR-activated macrophages (Figure 1B). ABCG1-facilitated cholesterol efflux to HDL would be expected to lead to decreased cholesterol mass accumulation in macrophages. In scrambled siRNA-treated, cholesterol-loaded, LXR-activated macrophages, HDL2-promoted cholesterol efflux reduced cellular cholesterol mass by ~25% (P<0.01) (Figure 1C). In contrast, HDL2 decreased cholesterol mass by ~12% (P<0.05) in macrophages transfected with siRNA targeting ABCG1. Together, these data indicate an essential role of ABCG1 in promoting cholesterol efflux to HDL and thereby regulating macrophage cholesterol content, especially after LXR activation.

To directly evaluate the role of ABCG4 in cholesterol efflux to HDL, we isolated macrophages from wild-type and ABCG4−/− mice. ABCG4 deficiency did not affect isotopic cholesterol efflux from macrophages to HDL2 with or without LXR activation (Figure 1D), indicating that ABCG4 does not have a role in the exchange or net efflux of macrophage cholesterol to HDL2. The small reduction of cholesterol efflux to HDL2 previously observed in macrophages treated with siRNA targeting ABCG4 might be caused by an off-target inhibition of ABCG1 translation.15 Together, these data
indicate that ABCG1 but not ABCG4 is essential for cholesterol efflux from LXR-activated macrophages.

Subcellular Localization of ABCG1 in Transfected HEK293 Cell

To understand how ABCG1 promotes cellular cholesterol efflux, we used ABCG1 antibodies to determine the cellular distribution of ABCG1 in transiently transfected 293 cells.9 Confocal immunofluorescence microscopy showed prominent plasma membrane as well as intracellular localization of ABCG1 (Figure 2A and 2B). Partial colocalization of ABCG1 with a Golgi marker, Golgi 97, and with transferrin was observed (Figure 2A and 2B), suggesting the presence of ABCG1 in Golgi and endocytic recycling compartments. In contrast, coimmunostaining with antibodies to calnexin, an endoplasmic reticulum marker, or antibodies to Lamp-1, a lysosomal marker, did not show significant colocalization with ABCG1 (not shown). The primary antibody to ABCG1 used in immunofluorescence studies showed a high specificity in Western analysis (Figure 2C). However, this antibody generated specific immunofluorescent signals only in 293 cells overexpressing ABCG1 but not in mouse peritoneal macrophages even after induction of ABCG1 expression by LXR activation (not shown), suggesting high specificity but low affinity of the antibody to ABCG1. We also tested GFP-tagged or Flag-tagged ABCG1 cDNA constructs. However, transient transfection of 293 cells with these tagged constructs failed to increase cholesterol efflux to HDL (Figure 2D), even though the expression of ABCG1-GFP or ABCG1-Flag was readily detectable by immunofluorescence microscopy (not shown). Attempts to insert a hemagglutinin tag on the predicted sixth extracellular/luminal loop again failed to generate a functional ABCG1 (not shown).

LXR Activation Increases ABCG1 at the Cell Surface in Macrophages

To evaluate the distribution of endogenous ABCG1 in mouse macrophages, we used subcellular fractionation by continuous sucrose density gradient centrifugation and Western analysis. We examined the distribution of ABCG1 in mouse macrophages with and without treatment with the LXR agonist, TO901317. Surprisingly, in basal macrophages, ABCG1 appeared to be primarily in intracellular fractions (Figure 3A), colocalizing with the Golgi marker, but not with the plasma membrane marker (β1-integrin) or endosomal marker (Rab5). In contrast, after LXR activation, ABCG1 distribution closely paralleled the distribution of β1-integrin (Figure 3B), indicating a predominant cell surface localization. To confirm the cell surface localization of ABCG1 in LXR-activated macrophages, we used limited proteolysis of cell surface proteins by treating intact cells with trypsin. ABCG1 protein levels in LXR-activated macrophages were substantially decreased by trypsin treatment at 37°C in a dose-dependent fashion (Figure 4A). The protein levels of β-actin, an intracellular protein, showed no change (Figure 4A).
4A). To confirm that trypsin activity was effectively blocked by trypsin inhibitors added at the end of the assay, we mixed lysates of control cells with lysates of trypsin-treated cells. ABCG1 protein levels showed no further change once the digestion was stopped by adding trypsin inhibitors (not shown). Therefore, the digestion of ABCG1 by trypsin occurred during the brief incubation of trypsin with intact cells and no further digestion occurred during washing and lysis of cells in the presence of trypsin inhibitors. These results are consistent with a predominant cell surface localization of ABCG1 in LXR-activated macrophages. We also examined the limited proteolysis of ABCG1 in intact cells by trypsin at room temperature ($\sim 25^\circ C$) using human macrophages derived from THP-1 cells in the presence or absence of LXR activation (Figure 4 and 4C). The lower temperature would likely limit any potential entry of trypsin into cells by endocytosis during incubation at $37^\circ C$. Unlike mouse peritoneal macrophage, these human macrophages expressed a moderate amount of ABCG1 protein even in the absence of LXR activation. LXR activation only moderately increased ABCG1 protein levels (Figure 4B and 4C). On trypsin treatment, ABCG1 protein levels in these LXR activated cells were reduced to a level similar to that of the control cells (without TO901317 treatment). Further, trypsin treatment did

Figure 2. ABCG1 is present at cell surface and in Golgi and endocytic recycling compartment in HEK293 cells. A, 293 cells transiently transfected with pcDNA/ABCG1 were examined using confocal immunofluorescence microscopy after cell permeabilization and immunostaining with anti-ABCG1 antibody (red) and anti-Golgi 97 antibody (green). B, Similar to (A), except that cells were incubated with Alexa488-labeled transferrin (green) at $37^\circ C$ for 15 minutes before immunostaining with anti-ABCG1 antibody (red). Note the specific staining of Golgi (A) and transferrin signal (B) in cells with or without ABCG1 overexpression. The images shown represent 1 $\mu$mol/L confocal slices. Scale bar, 10 $\mu$m/L. C, Western analysis of 293 cells transfected with empty vector (lane 1) or pcDNA3.1/ABCG1 (lane 2) with ABCG1 antibody. D, Cells transfected with the indicated plasmid construct were labeled with $[^3]H$-cholesterol in 10% fetal bovine serum and DMEM medium and cholesterol efflux to HDL2 for 4 hours was determined.

Figure 3. ABCG1 is present at the cell surface in LXR-activated macrophages. A, Subcellular fractionation of mouse macrophages in the basal state by a 0.58 to 1.1 mol/L continuous sucrose density gradient centrifugation was described in the Experimental Procedures and the results of Western analysis of each individual fraction are shown. B, Subcellular fractionation of LXR-activated mouse macrophages with a procedure similar to A.

Figure 4. LXR activation increases cell surface ABCG1 protein levels. A, Mouse macrophages treated with TO901317 (3 $\mu$mol/L, 16 hour) were incubated at $37^\circ C$ for 20 minutes with the indicated amount of trypsin followed by washing and cell lysis in the presence of trypsin inhibitors. Western analysis is shown. B, THP-1 macrophages treated with or without TO901317 (3 $\mu$mol/L, 16 hour) were incubated at $\sim 25^\circ C$ for 30 minutes with 0.5 mg/mL trypsin and shown is the Western analysis. C, ABCG1 protein levels normalized against $\beta$-actin in B were determined by densitometry.
ABCG1 overexpression in 293 cells increased cholesterol efflux in an ABCG1-Dependent Fashion in Macrophages

Efflux to HDL, LDL, and Cyclodextrin

LXR Activation Increases Cholesterol Efflux to HDL, LDL, and Cyclodextrin in an ABCG1-Dependent Fashion in Macrophages

We also used cell surface biotinylation of ABCG1 as an independent confirmation of the LXR-induced redistribution of ABCG1 to the cell surface. We used human THP-1 macrophages, because we were unable to successfully biotinylate mouse ABCG1 even under overexpression conditions. In the basal state, cell surface ABCG1 was barely detectable by biotinylation. However, LXR activation increased cell surface ABCG1 by ~6-fold (Figure 5A and 5B). These data provide an independent confirmation that LXR activation induces a redistribution of ABCG1 from intracellular sites to the plasma membrane.

Whereas the LXR activation in these studies relied on a synthetic LXR agonist, upregulation of ABCG1 expression in vivo by cholesterol loading is likely through generation of endogenous LXR ligands. Therefore, we also tested the effect of cholesterol loading on ABCG1 distribution in macrophages. In THP-1 macrophages, cholesterol loading by acetyl-LDL increased total ABCG1 protein mass by 1.7±0.24-fold, whereas the cell surface ABCG1, as determined by biotinylation, was increased by 2.6±0.31-fold (P<0.05). These results support the idea that LXR activation increases in the amount and a redistribution of ABCG1 to the cell surface as a physiological response to cholesterol accumulation.

LXR Activation Increases Cholesterol Efflux to HDL, LDL, and Cyclodextrin in an ABCG1-Dependent Fashion in Macrophages

ABCG1 overexpression in 293 cells increased cholesterol efflux not only to HDL but also to LDL and cyclodextrin, suggesting the possibility that ABCG1 activity increased the amount of cell surface cholesterol that was accessible to a variety of extracellular acceptors. To see whether similar results would be obtained in macrophages, we induced ABCG1 expression by LXR activation, then measured cholesterol efflux to the various acceptors. LXR activation significantly increased cholesterol efflux from macrophages to HDL, LDL, and cyclodextrin (Figure 6A). LXR activation also increased cholesterol efflux to apoA-I (Figure 6A), likely reflecting the upregulation of ABCA1 expression. To test the specific role of ABCG1, cholesterol efflux was determined from macrophages with or without suppression of ABCG1 expression by siRNA. Suppression of ABCG1 expression reduced cholesterol efflux from macrophages to HDL, LDL and cyclodextrin, but not apoA-1 (Figure 6B). These results suggest that LXR activation enhances ABCG1 expression and promotes cholesterol availability to a variety of lipoprotein acceptors and to cyclodextrin at the macrophage plasma membrane, but that this is not sufficient to promote cholesterol efflux to apoA-1.

Suppression of ABCG1 Expression Increases Cholesteryl Ester Formation and Decreases SREBP2 Target Gene Expression

To test the potential role of ABCG1 in regulation of ER cholesterol metabolism, we determined cholesteryl ester (CE) formation after acute suppression of ABCG1 expression in macrophages. When [14C]oleate was used as a substrate for ACAT-catalyzed CE formation, suppression of ABCG1 expression markedly increased CE formation in macrophages activated by 2 μmol/L TO901317 and loaded with cholesterol by incubation with acetyl-LDL (Supplement 1A). Intriguingly, this increased CE formation occurred in the absence of HDL-promoted cholesterol efflux. As a control, siRNA targeting ABCA7, another ABC transporter, showed no effect on CE formation (supplemental Figure 1A, see http://atvb.ahajournals.org). Decreased ABCG1 activity did not affect uptake of [14C]oleate by macrophages (not shown). The increased CE formation could be a result of an increased FC accessibility to ACAT as substrate in ER or a consequence of increased ACAT activity. Using real-time quantitative RT-PCR, we determined the mRNA levels of ACAT1, the major ACAT form in macrophase. ACAT1 mRNA levels did not show significant change on suppression of ABCG1 expression in macrophages (supplemental Figure IB). Similarly, downregulation of ABCG1 expression in mouse macrophages cholesterol loaded with acetyl-LDL but without TO901317 treatment also increased CE formation without alteration of ACAT1 mRNA levels (supplemental Figure IC and ID). Together, these findings suggest that ABCG1 activity alters the subcellular distribution of cholesterol, even in the absence of cholesterol efflux. Decreased ABCG1 activity may lead to an increased accumulation of FC in the ER, which subsequently serves as substrate for ACAT-catalyzed CE formation.

To further test this idea, we determined the expression of 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase and LDL receptor, 2 SREBP2 target genes. Down-regulation of ABCG1 expression by RNA interference decreased HMG CoA reductase mRNA levels by ~50% in cholesterol-loaded mouse peritoneal macrophages in the presence or absence of TO901317 (P<0.01). The mRNA levels of LDL receptor were already low in these cholesterol-loaded macrophages.
macrophages. Although reduced LDL receptor mRNA levels (≈35%) were detected on suppression of ABCG1 expression, the data did not reach statistical significance (not shown). Overall, the data are consistent with the idea that suppression of ABCG1 expression leads to an increased FC accumulation in the ER, which inhibits SREBP2 processing and activation while increasing CE formation by serving as substrates for ACAT.

Discussion

By using macrophages with targeted disruption of the ABCG1 or ABCG4 genes, we show that ABCG1 but not ABCG4 plays a pivotal role in macrophage cholesterol mass efflux to HDL after LXR activation. The results with ABCG4 are not surprising, because it is expressed primarily in the brain, where it could potentially play a role in promoting cholesterol efflux to HDL in the cerebrospinal fluid (CSF). ABCG1 had only a limited contribution to cholesterol efflux in the basal state without LXR activation or cholesterol loading, even though protein expression was readily detectable. A potential explanation for this finding was the novel observation that LXR activation in addition to increasing the amount of ABCG1 also resulted in a redistribution of ABCG1 from intracellular sites to the plasma membrane. Thus, LXRs in addition to inducing expression of target genes may lead to altered distribution of induced protein products within cells, leading to enhanced function. ABCG1 localizes in the plasma membrane of LXR-activated macrophages and increases the availability of cholesterol to a variety of particles that have direct capacity to accept cholesterol, but not to lipid-poor apoA-I. ABCG1 activity also appears to limit accumulation of FC in the ER, decreasing CE formation and expression of SREBP2 target genes, even in the absence of HDL. These findings provide a mechanism to explain the accumulation of CE in macrophage foam cells in ABCG1-deficient mice.

In LXR-activated or cholesterol-loaded macrophages, predominant cell surface localization of ABCG1 was shown by cellular fractionation, limited proteolysis and biotinylation experiments. Although LXR activation or cholesterol loading moderately increased total ABCG1 protein in human macrophages derived from THP-1 cells, surprisingly, a more substantial increase in ABCG1 protein at the cell surface was observed. Also subcellular fractionation suggested a major LXR-induced redistribution of ABCG1 from the Golgi to the

![Figure 6](http://atvb.ahajournals.org/)

**Figure 6.** LXR activation increases cholesterol efflux to HDL, LDL, and CD, but not apoA-I in macrophages in an ABCG1-dependent fashion. A, Mouse peritoneal macrophages were labeled with [3H]cholesterol in 10% FBS and DMEM medium in the presence or absence of 3 μmol/L TO901317 for 16 hours. Cholesterol efflux was initiated by adding HDL2 (25 μg/mL HDL protein), LDL (25 μg/mL LDL protein), or 2-hydroxypropyl-β-cyclodextrin (0.4 mmol/L) and proceeded at 37°C for 2 hours. B, Macrophages were transfected with 160 nmol scrambled or ABCG1 siRNA in the presence of 3 μmol/L TO901317 and labeled with [3H]cholesterol as described in the Experimental Procedures. Cholesterol efflux similar to A was determined. *P<0.05 for vehicle vs TO901317 in (A) or scrambled siRNA versus ABCG1 siRNA in (B) for the indicated acceptor.
plasma membrane (Figure 3). This finding could explain the lack of a contribution of ABCG1 to cholesterol efflux in the basal state, despite readily detectable ABCG1 in cell lysates (Figure 3). The mechanism for this increased ABCG1 presentation at the cell surface is unknown. Previous studies have shown several different isoforms of ABCG1 caused by alternative promoter usage or splicing,\textsuperscript{10,19} and some isoforms appeared to be more responsive to LXR activation than others.\textsuperscript{20} It is possible that different isoforms of ABCG1 have distinct preferences for cell surface localization. Alternatively, LXR activation may regulate ABCG1 cellular distribution by an undefined posttranscriptional mechanism.

The predominant cell surface localization of ABCG1 in LXR-activated macrophages argues for the plasma membrane as the primary site for ABCG1 to promote cellular cholesterol efflux to HDL. Conceivably, ABCG1 may translocate cholesterol from the inner to the outer leaflet of the plasma membrane, which subsequently is available for efflux, by diffusional or collisional mechanisms,\textsuperscript{6} to extracellular cholesterol acceptors.

LXR activation promoted macropage cholesterol efflux to HDL, LDL, and CD in an ABCG1-dependent fashion, consistent with our earlier findings in overexpressing 293 cells.\textsuperscript{9} Together these data suggest that ABCG1 activity redistributes cellular cholesterol to the cell surface, where it is accessible for removal by extracellular cholesterol acceptors. It is intriguing that this apparent increase in cell surface cholesterol content is not sufficient to promote cholesterol efflux to lipid-poor apoA-1. Whereas some have argued that ABCA1 acts to form cholester-ol-enriched microdomains that are subsequently solubilized by apoA-1, the findings with ABCG1 suggest that the ability of ABCA1 to bind apoA-1 may be key to its ability to promote lipid efflux to apoA-1. Interestingly, suppression of ABCG1 expression in cholesterol-loaded macrophages with or without T0901317 treatment markedly increased CE formation without change in ACAT mRNA level and reduced expression of SREBP-2 target genes. Importantly, these changes occurred in the absence of HDL in the medium. This suggests that activity of ABCG1 in the plasma membrane and possibly at intracellular sites promotes a redistribution of cholesterol away from regulatory pools in the ER. Surprisingly, we were not able to show colocalization of ABCG1 with ER markers. It is conceivable that activity of ABCG1 in plasma membrane or Golgi indirectly leads to a reduction in the ER cholesterol regulatory pool. Although the function of ABCG1 in plasma membrane is likely linked to HDL-mediated cholesterol efflux, its potential role in the Golgi is unknown. An interesting possibility is that Golgi ABCG1 is involved in the formation of cholesterol-rich lipid microdomains that are eventually transported to the cell surface. Our findings showing increased ACAT activity in ABCG1 deficient cells are consistent with the observation that ABCG1-deficient mice accumulate macropage foam cells in various organs\textsuperscript{11} but inconsistent with a recent report that ABCG1 overexpression increased CE accumulation in transfected BHK cells.\textsuperscript{12} Perhaps this discrepancy is related to very high overexpression of ABCG1 in the latter study. Increased CE accumulation in ABCG1-deficient macrophages is likely the result of increased free cholesterol in ER that is subsequently transferred to CE by ACAT activity.

References

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Supplement

A. Cholesterol esterification (nmol/mg cellular protein) for Scrambled, ABCA7, and ABCG1.

B. ACAT1/β-actin mRNA (arbitrary unit) for Scrambled, ABCA7, and ABCG1.

C. Cholesterol esterification (nmol/mg cellular protein) for Scrambled, ABCA7, and ABCG1.

D. ACAT1/β-actin mRNA (arbitrary unit) for Scrambled, ABCA7, and ABCG1.
Supplement.

Fig. 1. Suppression of ABCG1 expression increases CE formation in macrophage.

A. Mouse peritoneal macrophages were transfected with 160 nM scrambled siRNA, ABCA7 siRNA or ABCG1 siRNA in the presence of 3 μM TO901317 and cholesterol loaded with 50 μg/ml acetyl-LDL protein as described in the Experimental Procedures. CE formation was determined by pulse-labeling with [14C]oleate followed by lipid extraction, thin-layer chromatography fractionation and isotopic counting. B. Quantitative real-time RT-PCR analysis for ACAT1 and β-actin mRNA content in cells treated similarly as in A. C. Similar to A except that no TO901317 was used. D. Quantitative real-time RT-PCR analysis for ACAT1 and β-actin mRNA content in cells treated similarly as in C.