Recently, ATP-binding cassette transporter A1 (ABCA1), the defective molecule in Tangier disease, has been shown to stimulate phospholipid and cholesterol efflux to apolipoprotein A-I (apoA-I); however, little is known concerning the cellular cholesterol pools that act as the source of cholesterol for ABCA1-mediated efflux. We observed a higher level of isotopic and mass cholesterol efflux from mouse peritoneal macrophages labeled with $[^3$H]cholesterol/acyt low density lipoprotein (where cholesterol accumulates in late endosomes and lysosomes) compared with cells labeled with $[^3$H]cholesterol with 10% fetal bovine serum, suggesting that late endosomes/lysosomes act as a preferential source of cholesterol for ABCA1-mediated efflux. Consistent with this idea, macrophages from Niemann-Pick C1 mice that have an inability to exit cholesterol from late endosomes/lysosomes showed a profound defect in cholesterol efflux to apoA-I. In contrast, phospholipid efflux to apoA-I was normal in Niemann-Pick C1 macrophages, as was cholesterol efflux following plasma membrane cholesterol labeling. These results suggest that cholesterol deposited in late endosomes/lysosomes preferentially acts as a source of cholesterol for ABCA1-mediated cholesterol efflux.

Tangier disease (TD) is a rare condition associated with low levels of plasma high density lipoproteins (HDL) and accumulation of cholesterol and cholesteryl esters in macrophage foam cells in tonsils, spleen, and other tissues. The cellular defect in TD involves a marked decrease in the efflux of cholesterol and phospholipid to apoA-I, the major protein of HDL. Recently, TD was shown to be caused by mutations in the ABCA1 gene (24). While studying cholesterol efflux from macrophages from these animals have a profound defect in apoA-I-mediated cholesterol efflux (8–10), indicating that apolipoprotein-mediated cholesterol efflux is primarily mediated by ABCA1. In contrast, ABCA1 shows only slight interaction with HDL$_{3}$ and no interaction with HDL$_{2}$ (11). Cellular cholesterol efflux mediated by HDL is thought to involve a “passive” process that may be diffusion-mediated or may involve an interaction of HDL with scavenger receptor B-I (SR-BI) (12, 13).

ABCA1 is a full transporter with 12 membrane-spanning domains (5, 14). Transfection of ABCA1 in 293 cells reveals a predominant cell surface localization and suggests a direct interaction of ABCA1 with apoA-I (11). The primary activity of ABCA1 appears to be the translocation of phospholipid at the plasma membrane rather than direct interaction with cholesterol (15, 16). Phospholipid-apoA-I complexes formed by ABCA1 may promote cholesterol efflux in a secondary fashion perhaps involving distinct areas of the plasma membrane (15, 17). The nature of the cellular sites that donate cholesterol to these phospholipid-apoA-I complexes is poorly understood. This may involve specific plasma membrane domains that derive cholesterol from intracellular stores. The nature of intracellular sites that potentially donate cholesterol to the plasma membrane for ABCA1-mediated efflux is also unclear. Niemann-Pick C (I and II) molecules play an essential role in intracellular cholesterol trafficking, particularly in the exit of cholesterol from late endosomes/lysosomes (18–21). Earlier studies suggested a defect in cholesterol efflux to phospholipid vesicles in NPC1 fibroblasts (22), but the specific role of NPC1 in ABCA1-mediated cholesterol efflux has not been investigated.

The ABCA1 gene is up-regulated by cellular cholesterol loading (23). The mechanism of this effect is increased gene transcription mediated by the oxysterol-activated transcription factor liver X receptor (LXR) acting in a complex with retinoid X receptor (RXR) at a site on the proximal promoter of the ABCA1 gene (24). While studying cholesterol efflux from macrophages that had been treated with the LXR/RXR ligands 22(R)-hydroxycholesterol and 9-cis-retinoic acid to up-regulate ABCA1, we noticed a marked discrepancy between the magnitude of ABCA1 expression and the resulting stimulation of cholesterol efflux, depending on the method of cellular cholesterol labeling. This led to an investigation of the hypothesis that ABCA1 stimulates cholesterol efflux preferentially from a pool of cholesterol found in late endosomes/lysosomes. This hypothesis has been evaluated by comparing cholesterol efflux under different labeling conditions and supported by the demonstration of a profound defect in cholesterol efflux to apoA-I using macrophages from NPC1 mice.

**EXPERIMENTAL PROCEDURES**

**Ribonuclease Protection Assay**—Reverse transcription-polymerase chain reaction was used to obtain a fragment of the murine ABCA1

Received for publication, August 17, 2001, and in revised form, September 13, 2001
Published, JBC Papers in Press, September 14, 2001, DOI 10.1074/jbc.M107938200
cDNA. Murine ABCA1 and β-actin antisense riboprobes were prepared by in vitro transcription using murine ABCA1 β-actin cDNA plasmid constructs. The protected hybrid fragments for ABCA1 and β-actin were 290 and 160 base pairs, respectively. Ribonuclease protection assay was performed as described (25). In brief, 20 µg of total RNA were hybridized with 100,000 cpm ABCA1 and β-actin riboprobes. After hybridization in 30 µl of a buffer consisting of 40 mM PIPES, pH 6.0, 400 mM NaCl, 1 mM EDTA, and 80% formamide. The hybridization mixture was digested with 20 units of T<sub>2</sub> ribonuclease (Life Technologies, Inc.) at 37 °C for 1 h, extracted with phenol/chloroform, precipitated with ethanol, and dissolved in 5 µl of RNA loading buffer. The protected RNA hybrid fragments were resolved on a 6% polyacrylamide/urea gel and subjected to autoradiography.

**Immunoblot Analysis of ABCA1**—For immunoblot analysis of ABCA1, peritoneal macrophages were washed and scraped in PBS and lysed in 10 mM Tris-HCl, pH 7.3, 1 mM MgCl₂, and 0.5% Nonidet P-40 in the presence of protease inhibitors (0.5 µg/ml leupeptin, 1 µg/ml aprotinin, 1 µg/ml pepstatin A; Roche Molecular Biochemicals). Post-nuclear supernatants from cell lysates were prepared by centrifugation at 3000 g for 10 min. After removing the supernatants containing the exposed amounts of protein were reduced with 2-mercaptoethanol in gel loading buffer, fractionated by 7.5% SDS-polyacrylamide gel electrophoresis, and transferred to 0.22-µm nitrocellulose membranes. Immunoblotting was performed using an anti-ABCA1 antisera (Novus, Littleton, CO) and ECL (Amersham Pharmacia Biotech). The relative intensities of the bands were determined by densitometry (Molecular Dynamics, model 300A).

**Lipoprotein Isolation**—Human low density lipoprotein (LDL, 1.006–<d>1.063</d>) and high density lipoprotein (HDL, 1.063–<d>1.215</d>) were isolated by sequential ultracentrifugation. Acetyl LDL (AcLDL) was prepared as described (26). Apolipoprotein A-I (apoA-I) was purchased from Bionetics International (Sacoe, MD).

**Isolation and Culture of Mouse Peritoneal Macrophages**—Homozygous BALB/c mice, 5–10 weeks old, BALB/c xprl<sup>+/−</sup>/ mice (stock number 093092, Jackson Laboratory, Bar Harbor, ME). Mouse peritoneal macrophages were isolated from NPC1 and wild type (wt) littersmates by peritoneal lavage with PBS 3 days after intraperitoneal injection with 1 ml of 3.85% thioglycollate (Becton Dickinson, Sparks, MD). The isolated cells were plated onto 24-well plates and allowed to adhere by incubation for 4 h at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Life Technologies). After confirming the isolated cells by washing with PBS, the cells were further incubated for 2 days and then used for cholesterol labeling and efflux experiments.

**[3H]Cholesterol Labeling of Cells**—Mouse peritoneal macrophages were labeled with [3H]cholesterol carried by one of the three delivery agents to investigate cholesterol efflux from different pools: (a) AcLDL (late endosomes/lysosomes pool); (b) 10% FBS/DMEM (recycling endosomes/lysosomes pool); and (c) 5 µg/ml leupeptin (22(R)-hydroxycholesterol and 9-cis-retinoic acid). This treatment resulted in a marked up-regulation of ABCA1 mRNA (not shown) and protein levels (Fig. 1A) and an increase in cholesterol efflux (Fig. 1B) as anticipated (27). Surprisingly, the level of cholesterol efflux was about 2.5-fold higher in activated cells labeled with [3H]cholesterol AcLDL compared with cells labeled with [3H]cholesterol, 10% FBS (Fig. 1B, compare bars 4 and 2), despite comparable levels of ABCA1 expression (Fig. 1A; note that ABCA1 protein appears as a doublet for unknown reason). AcLDL is internalized by the scavenger receptor A and accumulates primarily in late endosomes and lysosomes (28), whereas the [3H]cholesterol, 10% FBS method appears to preferentially label recycling endosomes and the trans-Golgi network (29). These findings suggested the hypothesis that ABCA1 might preferentially stimulate cholesterol efflux from late endosomes/lysosomes rather than from cellular cholesterol pools labeled by [3H]cholesterol, 10% FBS.

To further explore this idea, cholesterol mass and isotopic efflux to apoA-I were measured in cells labeled with [3H]cholesterol, 10% FBS, with [3H]cholesterol/AcLDL, or with [3H]cholesterol/cyclohextrin. In the latter procedure, the cells were labeled briefly (15 min) with cyclohextrin:cholesterol (8:1, molar ratio), and the radiolabel is thought to reside mostly in the plasma membrane. After the labeling, the cells were incubated with 10% FBS for 24 h. The cells were then equilibrated overnight in DMEM, 0.2% BSA with or without the LXR/RXR ligands 22(R)-hydroxycholesterol and 9-cis-retinoic acid. After washing, the cells were used for efflux experiments. The efflux experiments were performed as described below. For procedure b, the cells were labeled with 1 µCi/ml [3H]cholesterol in 0.5 ml of DMEM supplemented with 10% FBS for 24 h. The cells were then equilibrated overnight in DMEM, 0.2% BSA with or without the LXR/RXR ligands 22(R)-hydroxycholesterol and 9-cis-retinoic acid. After washing, the cells were used for efflux experiments. The efflux experiments were performed as described below. For procedure c, the cells were washed with the ligands 22(R)-hydroxycholesterol and 9-cis-retinoic acid in DMEM, 0.2% BSA overnight to induce ABCA1. Then the medium was replaced by 5 mM methyl β-cycloexodrin:cholesterol at molar ratio 8:1 ([3H]cholesterol, 1 µCi/ml) for 15 min at 37 °C. After washing, the cells were used for efflux studies.
similar fashion to \([^{3}H]\)cholesterol/cyclodextrin-labeled cells. This finding could arise if the radiolabel was primarily present in pools of cholesterol inaccessible to ABCA1 (i.e. recycling endosomes) (29), whereas cholesterol mass efflux reflected efflux from the plasma membrane where cholesterol would be unlabeled by this method.

To further explore the hypothesis that late endosomes/lysosomes represent a preferred source of cholesterol for ABCA1-mediated cholesterol efflux, we next carried out efflux studies using macrophages from Niemann-Pick C1 mice, which have a defect in trafficking of cholesterol out of late endosomes (32). Cholesterol loading was carried out using \([^{3}H]\)cholesterol/AcLDL. Compared with macrophages from wild type mice, there was a profound decrease in cholesterol efflux to apoA-I in NPC1 macrophages, especially following induction of ABCA1 (Fig. 3A). Measurements of ABCA1 mRNA and protein revealed similar levels of induction in control and NPC1 macrophages (not shown). In earlier studies, Liscum et al. (22) reported that human NPC1 fibroblasts had a moderate defect in cholesterol efflux to small unilamellar vesicles; this was manifested as a delay in cholesterol efflux that became normal following longer incubation periods. However, a time course study revealed a profound 3–4-fold decrease in cholesterol efflux to apoA-I in NPC1 macrophages that was not ameliorated by prolonged incubation (Fig. 3B). If ABCA1 preferentially stimulates cholesterol efflux from late endosomes/lysosomes, then it might be anticipated that there would be a less pronounced defect in cholesterol efflux in NPC1 cells labeled with \([^{3}H]\)cholesterol, 10% FBS. Accordingly, using this labeling method, basal cholesterol efflux to apoA-I was similar in wild type and NPC1 cells, and efflux was only moderately decreased in NPC1 macrophages compared with wild type macrophages following LXR/RXR activation (Fig. 3C). Following plasma membrane labeling with \([^{3}H]\)cholesterol/cyclodextrin, there were identical levels of cholesterol efflux in NPC1 and wild type cells (Fig. 3D). These experiments suggest that both lysosomal and plasma membrane cholesterol pools serve as a source of cholesterol for ABCA1 and that the lysosomal pool requires the activity of the NPC1 molecule.

ABCA1 is thought to act as a phospholipid flipase at the plasma membrane (15, 16). This activity may lead to the formation of phospholipid-apoA-I complexes that secondarily stimulate cholesterol efflux from a distinct region of plasma membrane (15, 17). We measured phospholipid efflux to apoA-I in NPC1 and wt macrophages. Phospholipid efflux was stimulated following activation of LXR/RXR, but there was no defect in phospholipid efflux in NPC1 cells (Fig. 4). This indicates that the primary action of ABCA1, i.e. formation of phospholipid-apoA-I complexes, is intact in NPC1 cells.

Cholesterol efflux to HDL2 was also significantly decreased in NPC1 cells loaded with \([^{3}H]\)cholesterol/AcLDL (Fig. 5) or by the \([^{3}H]\)cholesterol, 10% FBS method (not shown). Because HDL2 does not interact with ABCA1 (11), this indicates a defect in cholesterol efflux via pathways not mediated by ABCA1. Interestingly, cholesterol efflux via HDL2 was also induced by LXR/RXR activation (Fig. 5). This suggests the presence of other LXR/RXR target genes in the HDL2-mediated efflux pathway. Because apolipoprotein E (apoE) was recently identified as an LXR/RXR target (33), we considered the possibility that increased cholesterol efflux might be due to increased

---

**FIG. 1.** Discrepancy between the magnitude of ABCA1 expression and the resulting cholesterol efflux with different cellular cholesterol labeling methods. wt peritoneal macrophages were \([^{3}H]\)cholesterol labeled by 10% FBS, DMEM (lanes 1 and 2) or 50 µg/ml AcLDL (lanes 3 and 4), with or without LXR/RXR ligands (22(R)-hydroxycholesterol and 9-cis-retinoic acid) treatment as described under “Experimental Procedures.” After equilibration in DMEM, 0.2% BSA, the cells were incubated with 10 µg/ml of apo-A-I for 4 h in DMEM, 0.2% BSA with or without the LXR/RXR ligands. A, Western blot of ABCA1 following SDS-polyacrylamide gel electrophoresis of cell lysates. Representative data are from one of two independent experiments. The values are the means ± S.D. (n = 3), *p < 0.01, bar 2 versus bar 4. **Cholesterol efflux** (B) was expressed as the medium \([^{3}H]\)cholesterol radioactivity (cells plus medium). Representative data are from one of three independent experiments. The values are the means ± S.D. (n = 3), *p < 0.01, AcLDL labeling versus 10% FBS and methyl-β-cyclodextrin (MβCD) labelings. 22ch, 22(R)-hydroxycholesterol; RA, 9-cis-retinoic acid.

**FIG. 2.** Isotopic and mass efflux of cholesterol from macrophages treated with the three different \([^{3}H]\)cholesterol labeling methods. wt mouse peritoneal macrophages in 6-well plates were \([^{3}H]\)cholesterol labeled by 10% FBS, AcLDL, or cyclodextrin and treated with or without the LXR/RXR ligands as described under “Experimental Procedures.” After equilibration in DMEM, 0.2% BSA, the cells were incubated with 10 µg/ml apo-A-I for 4 h. \([^{3}H]\)cholesterol efflux (A) was expressed as the medium \([^{3}H]\)cholesterol radioactivity as a percentage of total \([^{3}H]\)cholesterol radioactivity (cells plus medium). For cholesterol mass assay (B), cholesterol in the medium was first extracted and measured enzymatically. Representative data are from one of two independent experiments. The values are the means ± S.D. (n = 3), *p < 0.01, AcLDL labeling versus 10% FBS and methyl-β-cyclodextrin (MβCD) labelings. 22ch, 22(R)-hydroxycholesterol; RA, 9-cis-retinoic acid.
apoE synthesis by mouse peritoneal macrophages. However, LXR/RXR activation similarly increased cholesterol efflux to HDL2 in macrophages from apoE knock-out mice (not shown), eliminating this possibility. The ability of HDL2 to stimulate increased cholesterol efflux following LXR/RXR activation was also confirmed by cholesterol mass measurements, which indicated primarily an increase in HDL2 free cholesterol (Fig. 6). SR-BI neutralizing antibodies (34) did not affect cholesterol efflux mediated by HDL2 in either basal or LXR/RXR-stimulated conditions (not shown).

**DISCUSSION**

Our findings suggest that phospholipid-apoA-I complexes formed by ABCA1 initially stimulate cholesterol efflux from regions of the plasma membrane that preferentially utilize cholesterol deposited by modified LDL in late endosomes/lysosomes rather than cholesterol deposited at other intracellular sites. The equilibration of cell surface cholesterol with these intracellular sites requires the activity of the NPC1 molecule and could perhaps also involve trafficking of the ABCA1 molecule itself (35). ABCA1 is markedly less efficient in stimulating cholesterol efflux from cells that have been labeled with [3H]cholesterol, 10% FBS, which probably primarily labels recycling endosomes (29). A profound defect in ABCA1-mediated cholesterol efflux in NPC1 mutant macrophages may be an important factor explaining our recent observations showing an increase of atherosclerosis in apoE knock-out/NPC1 mutant mice, compared with apoE knock-out control mice.2

Massive cholesteryl ester accumulation in TD macrophages indicates that ABCA1 has an essential role in mediating cholesterol efflux to HDL in NPC1 and wild type peritoneal macrophages. NPC1 and wt peritoneal macrophages were [3H]cholesterol labeled by AcLDL (A and B), 10% FBS (C), and cycloextrin (D) with or without LXR/RXR ligand treatment as described under “Experimental Procedures.” After equilibration in DMEM, 0.2% BSA, the cells were incubated with 10 μg/ml of apoA-I for 4 h (A, C, and D) or different time points (B) in DMEM, 0.2% BSA with or without the ligands. Cholesterol efflux was expressed as the medium [3H]cholesterol radioactivity as a percentage of total [3H]cholesterol radioactivity (cells plus medium). Representative data are from one of three (A and C) or two (B and D) independent experiments. The values are the means ± S.D. (n = 3). *, p < 0.01, NPC1 versus wt. 22ch, 22(R)-hydroxycholesterol; RA, 9-cis-retinoic acid.

**FIG. 3.** ABCA1-mediated cholesterol efflux in NPC1 and wild type macrophages. NPC1 and wt peritoneal macrophages were [3H]cholesterol labeled by AcLDL (A and B), 10% FBS (C), and cycloextrin (D) with or without LXR/RXR ligand treatment as described under “Experimental Procedures.” After equilibration in DMEM, 0.2% BSA, the cells were incubated with 10 μg/ml of apoA-I for 4 h (A, C, and D) or different time points (B) in DMEM, 0.2% BSA with or without the ligands. Cholesterol efflux was expressed as the medium [3H]cholesterol radioactivity as a percentage of total [3H]cholesterol radioactivity (cells plus medium). Representative data are from one of three (A and C) or two (B and D) independent experiments. The values are the means ± S.D. (n = 3). *, p < 0.01, NPC1 versus wt. 22ch, 22(R)-hydroxycholesterol; RA, 9-cis-retinoic acid.

**FIG. 4.** ABCA1-mediated phospholipid efflux in NPC1 and wild type peritoneal macrophages. NPC1 and wt peritoneal macrophages were [3H]choline-labeled (1 μCi/ml of [3H]choline) and treated with or without the LXR/RXR ligands as described under “Experimental Procedures.” [3H]Phospholipid efflux was performed by incubation with 10 μg/ml of apoA-I for 4 h in DMEM, 0.2% BSA with or without the ligands. [3H]Phospholipid in the medium and cell lysates was determined following extraction in chloroform:methanol (2:1), and the radioactivity was determined by scintillation counting. [3H]Phospholipid efflux was expressed as the medium radioactivity as a percentage of total radioactivity (cells plus medium). Representative data are from one of two independent experiments. The values are the means ± S.D. (n = 3). 22ch, 22(R)-hydroxycholesterol; RA, 9-cis-retinoic acid.

**FIG. 5.** Cholesterol efflux to HDL2 in NPC1 and wild type peritoneal macrophages. NPC1 and wt peritoneal macrophages were [3H]cholesterol AcLDL-labeled and treated with or without the LXR/RXR ligands as described under “Experimental Procedures.” Efflux was performed by incubation with 15 μg/ml of HDL2 for 4 h in DMEM, 0.2% BSA with or without the ligands. Cholesterol efflux was expressed as the medium [3H]cholesterol radioactivity as a percentage of total [3H]cholesterol radioactivity (cells plus medium). Representative data are from one of three independent experiments. The values are the means ± S.D. (n = 3). *, p < 0.05, NPC1 versus wt. 22ch, 22(R)-hydroxycholesterol; RA, 9-cis-retinoic acid.

N. Sharma, G. Kuriakose, D. Zhang, I. Tabas, R. J. Deckelbaum, A. R. Tall, and C. L. Welch, submitted for publication.
Following entry of lipoprotein cholesterol into late endosomes and lysosomes, NPC1 has an essential role in allowing cholesterol to gain access to the ABCA1 efflux pool (Fig. 3). Liscum et al. (22) reported a delay in cholesterol efflux to unilamellar vesicles in fibroblasts from NPC1 patients. However, with time the efflux became normal. In contrast, the apoA-I stimulated cholesterol efflux in NPC1 mutant mouse macrophages was profoundly reduced at all time points (Fig. 3B). Recently, it has been shown that following labeling of NPC1 mutant Chinese hamster ovary cells with \(^{3}H\)-cholesterol ester LDL, early time points of cholesterol efflux to cyclodextrin show no or little defect (31, 42). However, after the initial appearance at the plasma membrane and subsequent internalization to an intracellular pool, cholesterol shows delayed trafficking back to the plasma membrane and poor activation of acyl-CoA-cholesterol acyltransferase in (ACAT) NPC1 mutant cells. This has led to the proposal that NPC1 acts on an intracellular pool of cholesterol that is derived from the plasma membrane and is in equilibrium with ACAT. Whether NPC1 is acting in late endosomes/lysosomes (43, 44) or on another pool of cholesterol (31), our studies suggest that this pool of cholesterol represents an important source of cholesterol for ABCA1-stimulated efflux.

Recently, Leventhal et al. (45) have found a defect in basal cholesterol efflux to apoA-I in acid sphingomyelinase-deficient macrophages. These studies suggest that endosomal/lysosomal sphingomyelin accumulation leads to cholesterol sequestration and, thus, defective cholesterol trafficking and efflux. The present findings extend these observations by providing direct evidence that the late endosomal/lysosomal cholesterol pool represents the preferred source of cholesterol for ABCA1-mediated efflux. Also, consistent with the present findings, Kojima et al. (46) recently reported that progesterone suppressed apoA-I-mediated cellular lipid release in human fibroblasts. Progesterone has been reported to sequester cholesterol in lysosomes and block cholesterol trafficking to plasma membrane similar to the effects of the NPC1 mutation (47). However, these earlier studies (45, 46) did not specifically compare cholesterol efflux from different cellular pools under conditions of high ABCA1 activities (i.e., following LXR/RXR activation and marked up-regulation of the ABCA1 (Fig. 1)) and did not specifically evaluate the effect of the NPC1 molecule in ABCA1-mediated cholesterol efflux as in the present study.

Our findings that NPC1 mutant macrophages have a prominent defect in cholesterol efflux from the late endosomal/lysosomal pool but only a moderate decrease in efflux from recycling endosomal pool (Fig. 3, A and C) may explain some earlier in vivo work showing that de novo synthesized cholesterol or cholesterol entering cells through the HDL/SR-BI pathway can be metabolized and excreted normally, whereas LDL-derived cholesterol becomes sequestered in the lysosomal compartment and is metabolically inactive in NPC1 mutant mice (48, 49) and in NPC1 patients (50). Labeling of cholesterol by DMEM, 10% FBS might mimic more closely the trafficking of de novo synthesized cholesterol or cholesterol derived from the HDL/SR-BI pathway, whereas labeling by AcLDL is similar to LDL cholesterol trafficking to lysosomes.

The HDL2-mediated cholesterol efflux pathway, distinct from that mediated by ABCA1 (12, 11), was also defective in NPC1 cells. An intriguing, unexpected observation was the finding that macrophage cholesterol efflux to HDL2 was increased by treatment with LXR/RXR activators (Fig. 5), suggesting a novel efflux process unrelated to ABCA1, SR-BI, or apoE. The mechanism of HDL2-mediated cholesterol efflux appears quite distinct from apoA-I-mediated cholesterol efflux. Thus, apoA-I binds and interacts with ABCA1 to mediate cho-
HDL2 levels, whereas HDL3 levels are relatively constant in differences in overall HDL levels between different subjects, cholesterol efflux, whereas HDL2 is inactive in this regard (11). These findings suggest that there is a novel molecular target of LXR/ RXR activation in the cholesterol efflux pathway to HDL2. This may well have physiological importance because differences in overall HDL levels between different subjects, such as male/female differences, are primarily due to different HDL2 levels, whereas HDL3 levels are relatively constant in the population (51).

REFERENCES

1. Assmann, G., von Eckardstein, A., and Brewer, H. B. (2001) The Metabolic and Molecular Domains of HDL. Annu. Rev. Med. 52, 179–194.

2. Francis, G. A., Knopp, R. H., and Oram, J. F. (1995) J. Clin. Invest. 95, 78–87.

3. (2000) Biochemistry 39, 14113–14120.

4. Brewer, H. B., Jr. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 1813–1821.

5. Brooks-Wilson, A., Marciel, M., Clee, S. M., Zhang, L. H., Roop, K., van Dam, M., Yu, L., Brewer, C., Collins, J. A., Molfatianu, H. O., Louhiche, G., Ono, F., Pichler, K., and Ashburner-Excalfin, K. J., Senseen, C. W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Pignone, S., Kastelein, J. J., Hayden, M. R., et al. (1999) Nat. Genet. 23, 396–405.

6. Rust, S., Rosier, M., Funke, H., Reali, J., Amoura, Z., Piette, J. C., Deleuze, J. F., Brewer, H. B., Jr., Newburn, T., and Voko, M. N. (1999) Nat. Genet. 23, 396–405.

7. Remaley, A. T., Rosier, M., Funke, H., Reali, J., Amoura, Z., Piette, J. C., Deleuze, J. F., Brewer, H. B., Jr., Newburn, T., and Voko, M. N. (1999) Nat. Genet. 23, 396–405.

8. Assmann, G., von Eckardstein, A., and Brewer, H. B., Jr. (1997) J. Clin. Invest. 100, 41316–41320.

9. Carsteanu, I., Comly, M., Cooney, A., Martinez, P. D., Bluett, A., S. L., and Vanier, M. T., et al. (1999) Mol. Genet. Metab. 68, 1–13.

10. Carsteanu, I., Comly, M., Cooney, A., Martinez, P. D., Bluett, A., S. L., and Vanier, M. T., et al. (1999) Mol. Genet. Metab. 68, 1–13.

11. Stark, D. L., Wang, N., Xiao, X., and Tall, A. R. (2001) J. Biol Chem. 276, 25297–25296.

12. Feinberg, S., and Maxfield, F. R. (1999) J. Biol. Chem. 274, 27590–27596.

13. Yoshimura, Y., and Yamauchi, T. (2000) J. Biol. Chem. 275, 27177–27174.

14. Saito, M., Kato, Y., and Nakamura, Y. (2000) J. Biol. Chem. 275, 27177–27174.

15. Naureckiene, S., Sleat, D. E., Lackland, H., Fensom, A., Vanier, M. T., Wadalia, R., Jadot, M., and Lobel, P. (2000) Science 289, 2258–2263.

16. Langmann, T., Ruggiero, R. M., and Faust, J. R. (1999) J. Biol. Chem. 274, 1625–1636.

17. Fielding, P. E., Nagan, K., Hakamata, H., Chumski, G., and Fielding, C. J. (1997) J. Biol. Chem. 272, 20982–20985.

18. Remaley, A. T., Rust, S., Funke, H., Reali, J., Amoura, Z., Piette, J. C., Deleuze, J. F., Brewer, H. B., Jr., Newburn, T., and Voko, M. N. (1999) Nat. Genet. 23, 396–405.

19. Pentchev, P. G., Comly, M., Krizman, D. B., Nagle, J., Polymeropoulos, M. H., Sturley, S. L., Assmann, G., van Eckardstein, A., Brewer, H. B., Jr., and Vanier, M. T. (1997) Science 275, 228–231.

20. Carsteanu, I., Comly, M., Krizman, D. B., Nagle, J., Polymeropoulos, M. H., Sturley, S. L., Assmann, G., van Eckardstein, A., Brewer, H. B., Jr., and Vanier, M. T. (1997) Science 275, 228–231.
Preferential ATP-binding Cassette Transporter A1-mediated Cholesterol Efflux from Late Endosomes/Lysosomes
Wengen Chen, Yu Sun, Carrie Welch, Anna Gorelik, Andrew R. Leventhal, Ira Tabas and Alan R. Tall

J. Biol. Chem. 2001, 276:43564-43569.
doi: 10.1074/jbc.M107938200 originally published online September 14, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107938200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 47 references, 30 of which can be accessed free at http://www.jbc.org/content/276/47/43564.full.html#ref-list-1