ABCA1 redistributes membrane cholesterol independent of apolipoprotein interactions

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Abstract **ATP binding cassette transporter A1 (ABCA1) mediates the transport of phospholipids and cholesterol from cells to lipid-poor HDL apolipoproteins. Cholesterol loading of cells induces ABCA1, implicating cholesterol as its major physiologic substrate. It is believed, however, that ABCA1 is primarily a phospholipid transporter and that cholesterol efflux occurs by diffusion to ABCA1-generated phospholipid-rich apolipoproteins. Here we show that overexpression of ABCA1 in baby hamster kidney cells in the absence of apolipoproteins redistributed membrane cholesterol to cell-surface domains accessible to treatment with the enzyme cholesterol oxidase. The cholesterol removed by apolipoprotein A-I (apoA-I), but not by HDL phospholipids, was derived exclusively from these domains. ABCA1 overexpression also increased cholesterol esterification, which was prevented by addition of apoA-I, suggesting that some of the cell-surface cholesterol not removed by apolipoproteins is transported to the intracellular esterifying enzyme acyl-CoA:cholesterol acyltransferase. ABCA1 expression was essential for cholesterol efflux even when apolipoproteins had already acquired phospholipids during prior exposure to ABCA1-expressing cells. These studies show that ABCA1 redistributes cholesterol to cell-surface domains, where it becomes accessible for removal by apolipoproteins, consistent with a direct role of ABCA1 in cholesterol transport.**—Vaughan, A. M., and J. F. Oram. **ABCA1 redistributes membrane cholesterol independent of apolipoprotein interactions.** ***J. Lipid Res.*** **2003.** 44: **1373–1380.**

Supplementary key words ATP binding cassette transporter A1 • high density lipoprotein • acyl-CoA:cholesterol acyltransferase • cholesterol efflux • cholesterol esterification

Population studies have shown an inverse relationship between plasma HDL levels and risk for cardiovascular disease, implying that factors associated with HDL metabolism are atheroprotective. One of these factors is a cell membrane ATP binding cassette (ABC) transporter called ABCA1, which mediates the transport of excess cholesterol from cells to HDL apolipoproteins (1). *ABCA1* mutations can cause Tangier disease (2–6), a severe HDL deficiency syndrome characterized by cholesterol deposition in tissue macrophages and prevalent atherosclerosis (7–10). Genetic manipulations of ABCA1 expression in mice also affect plasma HDL levels and atherogenesis (11–15).

ABCA1 mediates the transport of both phospholipid and cholesterol to lipid-poor apolipoproteins by mechanisms still poorly understood. Cholesterol loading of cells markedly induces ABCA1 (6, 16), consistent with cholesterol being the physiologic substrate for ABCA1. Several studies, however, have suggested that ABCA1 is primarily a phospholipid transporter and promotes cholesterol efflux only after generating phospholipid-rich apolipoprotein acceptors for cholesterol that diffuses from cells (17–19). Other studies have provided evidence that apolipoproteins simultaneously remove phospholipids and cholesterol from cells by microsolubilizing plasma membrane lipid domains formed by ABCA1 (20, 21).

As one approach to distinguishing between these mechanisms, we examined whether ABCA1 expression modulates the distribution of cholesterol between cellular compartments. We show that induction of ABCA1 redistributes cholesterol to cell-surface domains accessible for removal by apolipoproteins. We also present evidence that ABCA1 expression is required for acute transport of cholesterol from cells regardless of whether the apolipoproteins had already acquired phospholipids by the ABCA1 pathway. These findings support the model that apolipoproteins simultaneously solubilize phospholipids and cholesterol from cell-surface domains generated by ABCA1.

EXPERIMENTAL PROCEDURES

HDL, trypsinized HDL, and apolipoprotein A-I

HDL was prepared by sequential ultracentrifugation in the density range 1.125–1.21 g/ml and was depleted of apolipoprotein E (apoE) and apoB by heparin-agarose chromatography

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Abbreviations: ABCA1, ATP binding cassette transporter A1; apoA-I, apolipoprotein A-I; BHK, baby hamster kidney; TrHDL, trypsinized HDL.

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(22). Trypsinized HDL (TrHDL) was prepared as previously described (23) by treating HDL with trypsin for 30 min at 37°C at an HDL-trypsin protein ratio of 40:1. This procedure digests ~15% of the total HDL protein content of HDL without disturbing its lipid composition. ApoA-I was purified from HDL, delipidated, and labeled with ^{125}I as described previously (22).

Cell culture

All cell culture incubations were performed at 37°C in a humidified 5% CO_2 incubator. Baby hamster kidney (BHK) cells were obtained from ATCC (Manassas, VA). BHK cells expressing human ABCA1 were generated using the mifepristone-inducible GeneSwitch system (Invitrogen, Carlsbad, CA). Cells were transfected initially with the pSwitch plasmid using Fugene6 (Roche Molecular Biochemicals). Clonal lines were isolated and then transfected with pGene/V5-His/*lacZ* and assayed for β-galactosidase activity with the β-Gal assay kit (Invitrogen). Clonal pSwitch lines that gave the highest mifepristone-induced β-galactosidase activity were then transfected with linearized pGene/V5-HisA containing a cDNA encoding the full-length open reading frame of human *ABCA1*. Mock-transfected BHK cells were derived from the same pSwitch clonal line transfected with a plasmid lacking the *ABCA1* cDNA. Cells were grown and maintained in DMEM containing 10% fetal bovine serum until experimental treatments. Unless indicated otherwise, ABCA1 was induced by incubating cells for 18–20 h in DMEM with 1 mg/ml fatty acid-free bovine serum albumin (BSA) and 10 nM mifepristone.

Plasma membrane cholesterol was depleted by treating cells for 30 min at 37°C with 20 mM methyl-β-cyclodextrin. With ABCA1-transfected cells, this treatment reduced apoA-I-mediated [^3H]cholesterol efflux by ~75% while having no effect on [^3H]choline-labeled phospholipid efflux.

Lipid efflux and cholesterol transport and esterification

To radiolabel cellular cholesterol to equilibrium, [^3H]cholesterol (0.5 μCi/ml, 40–60 Ci/mmol, Amersham Pharmacia Biotech) was added to the 10% serum medium 1–2 days prior to mifepristone treatment. To selectively label the plasma membrane, cells were incubated for 1 h with DMEM-BSA containing 2 μCi/ml [^3H]cholesterol and then washed three times with phosphate-buffered saline (PBS) containing 1 mg/ml BSA. To radiolabel cellular phospholipids, 1 μC_i/ml [^3H]choline chloride (75–85 C_i/mmol; Amersham Pharmacia Biotech) was added during the mifepristone incubations, and cells were washed three times with PBS-BSA.

To measure lipid efflux, cells were incubated with DMEM-BSA with or without 10 μg/ml apoA-I for 2–6 h at 37°C and chilled on ice, and the medium was collected and centrifuged to remove detached cells. For cholesterol efflux, the medium was counted for ^3H, and the cells were assayed for free and esterified [^3H]cholesterol after isolation by thin-layer chromatography (TLC) (24). For phospholipid efflux, medium and cellular choline-labeled phospholipids were extracted in $CHCl_3$-CH_3OH and assayed for ^3H radioactivity (24). ApoA-I-mediated lipid efflux was calculated as the percent total [^3H]lipid released into the medium after subtraction of values obtained in the absence of apoA-I.

To measure cell-surface cholesterol redistribution, cells were washed once with PBS and then incubated with 1 U/ml cholesterol oxidase (Calbiochem) in DMEM at 37°C for 10 min or at 15°C for 60 min (25, 26). Cells were then washed twice with PBS, cellular lipids were extracted, and [^3H]cholesterol and [^3H]cholestenone were measured after isolation by TLC. Incorporation of oleate into cholesteryl esters was assayed by incubating cells with [^{14}C]oleate (56 mCi/ml, Amersham Pharmacia Biotech) and measuring cellular ^{14}C radioactivity in TLC-isolated cholesteryl esters (24).

Cell-surface binding of apoA-I

Cells were incubated for 2 h at 0°C with HEPES-buffered DMEM-BSA containing 1 μg/ml ^{125}I-apoA-I plus or minus 200 μg/ml unlabeled apoA-I and then washed at 0°C twice with PBS-BSA and twice with PBS (24, 27). Cell-associated radioactivity and cell protein were measured after digestion in 0.2 N NaOH. Results are expressed as ng of ^{125}I-apoA-I per mg of cell protein after subtraction of values in the presence of unlabeled HDL.

Immunoblotting

Control and ABCA1-transfected BHK cells were solubilized in 50 mM Tris buffer containing 1% SDS, 0.1 M mercaptoethanol, and 0.5 mM EDTA, and proteins were resolved by 6% polyacrylamide gel electrophoresis. Proteins were transblotted onto nitrocellulose, and ABCA1 was identified with a C-terminal antibody using an enhanced chemiluminescence detection assay (28).

Statistics

Data were analyzed by paired Student's *t*-test to determine significance. Each experiment shown is representative of at least three similar experiments.

RESULTS

Induction of ABCA1 alters cellular cholesterol distribution

To examine the effects of ABCA1 expression on cell cholesterol homeostasis, we stably transfected BHK cells with an ABCA1 cDNA that is inducible by treating cells with mifepristone. As controls, we transfected cells with the same vectors lacking the ABCA1 cDNA insert (mock-transfected). This transfection system allowed us to induce ABCA1 to high levels for finite periods of time and minimize potential cytotoxic effects of long-term overexpression of ABCA1 in membranes.

Mifepristone-treated, mock-transfected BHK cells had essentially no detectable ABCA1 protein and apoA-I-mediated cholesterol and phospholipid efflux activities (**Fig. 1**). In contrast, mifepristone treatment of ABCA1-transfected cells caused a marked increase in ABCA1 levels and apoA-I-mediated cholesterol and phospholipid efflux (Fig. 1). Based on immunoblot comparisons, the membrane content of ABCA1 in these BHK transfectants was ~10-fold higher than that in cAMP-induced murine macrophages. An increase in cholesterol efflux occurred after only 6 h of mifepristone treatment (data not shown). ABCA1 induction also led to an increase in high-affinity binding of apoA-I to the cell surface (Fig. 1). Mock- and ABCA1-transfected cells treated with mifepristone for 0 to 24 h had the same microscopic appearance.

We tested the possibility that ABCA1 expression alone could alter cellular cholesterol homeostasis by radiolabeling cellular cholesterol, treating cells for 24 h with increasing concentrations of mifepristone, and measuring labeled cholesterol distribution between cellular pools. The relative distribution of cell-surface free cholesterol was determined by treating cells for 10 min at 37°C with the enzyme cholesterol oxidase and assaying for cellular cholestenone. With noninduced cells, only 7–10% of the labeled cholesterol was converted to cholestenone (**Fig. 2**),

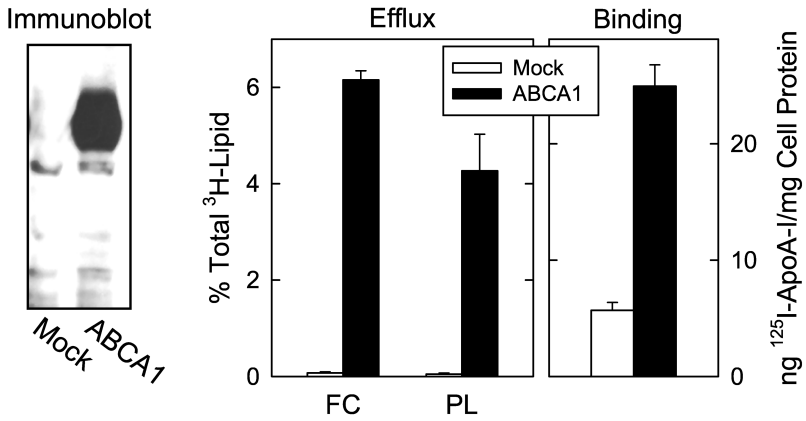

Fig. 1. ATP binding cassette transporter A1 (ABCA1) protein levels and activity in mock- and ABCA1-transfected baby hamster kidney (BHK) cells. For immunoblots, transfected BHK cells were treated with 10 nM mifepristone for 20 h, and ABCA1 protein levels were measured in equal amounts of cell protein (40 μg) as described in Experimental Procedures. For lipid efflux, [^{3}H]cholesterol- and [^{3}H]choline-labeled cells were treated with 10 nM mifepristone for 18 h, and [^{3}H]cholesterol (FC) and [^{3}H]choline-labeled phospholipid (PL) efflux was measured after 4 h incubations with 10 μg/ml apolipoprotein A-I (apoA-I). Results are the mean ± SD of triplicates expressed as the percent of total radiolabeled cholesterol or phospholipid (medium plus cell) released into the medium. For the binding assay, mifepristone-treated cells were incubated for 2 h at 0°C with 1 μg/ml ^{125}I-apoA-I minus or plus 200 μg/ml unlabeled apoA-I, and high-affinity binding was determined. Each value is the mean ± SD of triplicates.

indicating that most of the cellular cholesterol was in a form that was inaccessible to cholesterol oxidase. Treatment of mock transfectants with mifepristone had no effect on this fraction of oxidizable cholesterol. In contrast, mifepristone treatment increased the fraction of oxidase-accessible cholesterol in ABCA1 transfectants to over 20% (Fig. 2). When apoA-I was added to the medium for 4 h immediately before the oxidase treatment, the cholesterol removed from cells was derived exclusively from the oxidase-accessible pool (Fig. 2). These results show that ABCA1 expression redistributes cholesterol to cell-surface domains accessible to exogenously added enzyme and apoA-I.

We compared the abilities of apoA-I and TrHDL to deplete cellular pools of cholesterol to test if phospholipid-rich HDL particles could also remove this ABCA1-generated oxidase-accessible cholesterol. Because of digested exchangeable apolipoproteins, TrHDL can only promote cholesterol efflux by ABCA1-independent diffusion mechanisms (2, 23). Whereas apoA-I removed cholesterol only from ABCA1-expressing cells, TrHDL promoted cholesterol efflux from both mock- and ABCA1-transfected cells (**Fig. 3A**). TrHDL decreased radiolabled cholesterol in pools resistant to oxidase treatment, while apoA-I had no effect on this cholesterol pool size (Fig. 3B). Conversely, apoA-I depleted oxidase-accessible cholesterol in the ABCA1-expressing cells, while TrHDL had no significant effect on this cholesterol fraction in either cell line (Fig. 3C). These results show that HDL phospholipids are unable to promote cholesterol efflux from the ABCA1-generated pool of cell-surface cholesterol that is removable by apoA-I.

To confirm that the ABCA1-redistributed cholesterol was localized to the cell surface, we measured the fraction of oxidase-accessible cholesterol after cells were treated with enzyme at 15°C, a temperature that prevents endocytosis of molecules and inhibits cholesterol trafficking (25, 29), thus limiting the enzyme reaction to cell-surface cholesterol. Because of a slower conversion rate, we extended the treatment time to 1 h. This low-temperature assay converted 9.9 ± 1.7% and 19.2 ± 2.2% (n = 3) of labeled free cholesterol to cholestenone in mifepristone-treated mock and ABCA1 transfectants, respectively. Addition of apoA-I for 4 h prior to oxidase treatment reduced this fraction in ABCA1 transfectants to 12.1 ± 0.05%, whereas it had no effect in mock transfectants (8.9 ±1.5%). ApoA-I had no effect on the fraction of free cholesterol resistant to oxidase treatment. These results are very similar to those shown in Figs. 2 and 3, implying that both the 15°C and 37°C oxidase assays are measuring the same fraction of cholesterol and that this fraction resides on the cell surface.

The above experiments were performed with cells labeled to equilibrium with [^{3}H]cholesterol by including tracer in the growth medium for 24 h prior to the mifepristone treatment. To determine if ABCA1 redistributes plasma membrane cholesterol during steady-state levels of expression, we first treated cells with mifepristone, labeled plasma membrane cholesterol by incubating cells with [^{3}H]cholesterol for 1 h, chased cells for 5 h without or with apoA-I, and measured the fraction of cholesterol accessible to cholesterol oxidase. With mock transfectants, only about 7% of the labeled cholesterol was oxidase accessible (**Fig. 4A**), indicating that most of the label was hidden from the enzyme even after this brief labeling/chase protocol. As expected, apoA-I had no effect on this distribution. With ABCA1-expressing cells, however, over 30% of the labeled cholesterol was oxidase accessible, and apoA-I removed cholesterol almost exclusively from this pool

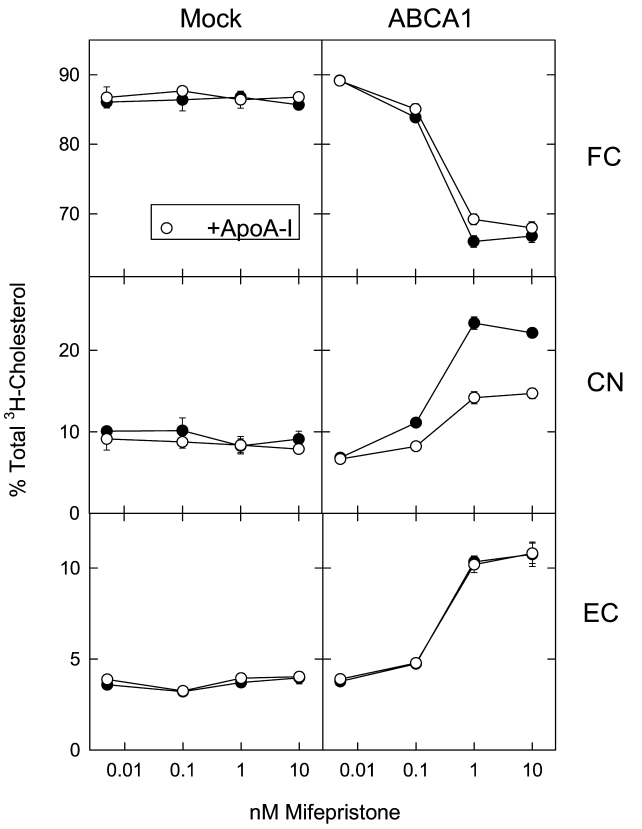

Fig. 2. Change in cellular cholesterol distribution in response to ABCA1 induction. [^{3}H]cholesterol-labeled cells were incubated for 20 h with the indicated concentration of mifepristone, incubated for 4 h with the same media without (closed circles) or with (open circles) 10 μg/ml apoA-I, treated with 1 U/ml cholesterol oxidase for 10 min at 37°C, and assayed for medium (not shown) and cell free [^{3}H]cholesterol (FC), cell [^{3}H]cholestenone (CN), and cell esterified [^{3}H]cholesterol (EC). Results are the mean ± SD of triplicates expressed as the percent of total (medium plus cell) radiolabeled cholesterol (FC, CN, and EC).

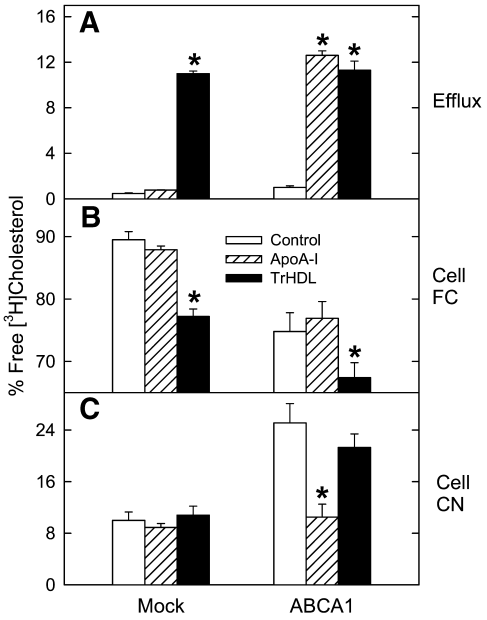

Fig. 3. ApoA-I- and trypsinized HDL (TrHDL)-mediated cholesterol efflux from cellular domains. [^{3}H]cholesterol-labeled cells were incubated for 22 h with 10 nM mifepristone, incubated for 6 h with the same media without (Control) or with 10 μg/ml apoA-I or 50 μg/ml TrHDL, treated with 1 U/ml cholesterol oxidase for 10 min at 37°C, and assayed for medium (A, Efflux), cell free [^{3}H]cholesterol (B, FC), and cell [^{3}H]cholestenone (C, CN). Results are the mean ± SD of triplicates expressed as the percent of total radiolabeled free cholesterol (plus cholestenone). *$P < 0.02$ versus control.

(Fig. 4B). Thus, cholesterol was redistributed in ABCA1-expressing cells even when plasma membrane cholesterol was radiolabeled by a brief pulse incubation.

We examined the rate at which ABCA1 redistributes cholesterol by first incubating mifepristone-treated cells with apoA-I to deplete oxidase-accessible cholesterol and then measuring the reappearance of this cholesterol pool during incubations without apoA-I. With ABCA1 transfectants, there was an almost linear increase in oxidase-accessible cholesterol during the first 6 h after removal of apoA-I (Fig. 4C). These cells replenished this cholesterol pool at a rate of 1.5% of their total cholesterol per h (~0.6 μg/mg cell protein/h).

Induction of ABCA1 increases cholesterol esterification

Inducing ABCA1 consistently increased the fraction of cholesterol that was esterified (Fig. 2), suggesting that ABCA1 also promotes flux of cholesterol into the substrate pool for acyl-CoA:cholesterol acyltransferase (ACAT). In the above experiment, apoA-I had no effect on this fractional esterification because it was added after these esters were formed. To determine if apoA-I affects this flux, we labeled plasma membrane cholesterol for 1 h, chased cells without or with apoA-I for 6 h, and measured the labeled free and esterified cholesterol content. After the chase without apoA-I, the free [^{3}H]cholesterol content of mock and ABCA1 transfectants was nearly identical (**Fig. 5A**). Only a small fraction of the radiolabeled cholesterol (<5%) became esterified after these short-term labeling and chase incubations, but the ABCA1 transfectants had a significantly higher content of [^{3}H]cholesteryl esters (Fig. 5B). These results are consistent with ABCA1 promoting flux of cholesterol into the ACAT substrate pool. Addition of apoA-I, which had no effect with mock transfectants, reduced both the free and esterified cholesterol content of the ABCA1-expressing cells (Fig. 5A, B). These data imply that the increased cholesteryl esters in ABCA1-expressing cells are derived from the free cholesterol pool that is removable by apoA-I.

As another index of the ACAT substrate pool size, we incubated cells for 6 h with mifepristone plus [^{14}C]oleate and measured incorporation of radiolabel into esterified cholesterol. ABCA1 transfectants incorporated more oleate into cholesterol esters than mock transfectants did, and including apoA-I in the medium eliminated this difference (Fig. 5C). There was no difference in incorporation of oleate into triglycerides under any of these incubation conditions (data not shown). Thus, ABCA1-expressing cells contained a larger cholesterol substrate pool for ACAT than did cells lacking ABCA1, and apoA-I depleted this pool.

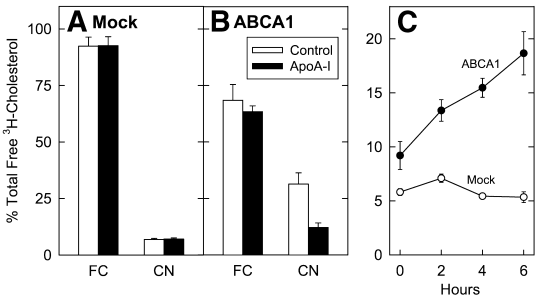

Fig. 4. ABCA1-promoted transport of plasma membrane cholesterol to the cell surface. A, B: Cells were treated with 10 nM mifepristone for 20 h, radiolabeled with [^{3}H]cholesterol for 1 h, incubated for 4 h with mifepristone without (Control) or with 10 μg/ml apoA-I, and treated with cholesterol oxidase for 10 min at 37°C. Wells were assayed for medium (not shown) and cell free [^{3}H]cholesterol (FC) and cell [^{3}H]cholestenone (CN). C: [^{3}H]cholesterol-labeled cells were incubated for 18 h with 10 nM mifepristone plus 10 μg/ml apoA-I, incubated for the indicated times without apoA-I, treated with cholesterol oxidase, and assayed for free [^{3}H]cholesterol (not shown) and [^{3}H]cholestenone. Results are the mean ± SD of triplicates expressed as percent of total radiolabeled free cholesterol (plus cholestenone).

ABCA1 expression is required for apolipoprotein-mediated cholesterol efflux

Previous studies have suggested that apoA-I removes cellular phospholipids and cholesterol by a two-step mechanism, whereby phospholipids are first removed by the ABCA1 pathway and cholesterol is then acquired by ABCA1-independent processes (17–19). We used a pulse-chase protocol to see if this mechanism occurs with our ABCA1 transfectants. We incubated unlabeled mock and ABCA1 transfectants for 4 h with apoA-I-containing medium, transferred these conditioned media to cholesterol-labeled cells, and measured cholesterol efflux during 2 h incubations. If apoA-I promotes cholesterol efflux by a two-step mechanism, conditioned medium from ABCA1 transfectants should stimulate cholesterol efflux from both mock and ABCA1 transfectants because of its increased phospholipid content. To ensure that apoA-I would not become saturated with cholesterol during the pulse incubations, we depleted membrane cholesterol from a set of cells by cyclodextrin treatment (30) under conditions that reduce apoA-I-mediated cholesterol efflux by more than 70% while having no effect on phospholipid efflux.

Results show that conditioned medium from ABCA1 transfectants was no more effective than medium from mock transfectants in promoting cholesterol efflux from mock transfectants, regardless of whether cells were pretreated with cyclodextrin (**Fig. 6A**). Thus, first exposing apoA-I to ABCA1-expressing cells did not increase its ability to remove cholesterol from cells lacking ABCA1. In contrast, all of these different conditioned media stimulated cholesterol efflux from ABCA1 transfectants (Fig. 6B). These results show that expression of ABCA1 was required for promotion of cholesterol efflux by apoA-I.

DISCUSSION

Although cholesterol loading of cells induces ABCA1 expression, it is believed that ABCA1 is largely a phospholipid transporter and promotes cholesterol efflux only after generating phospholipid-rich apolipoprotein acceptors (17–19). Here we show that ABCA1 intrinsically redistributes cholesterol to cell-surface domains that are accessible to apolipoproteins. In addition, we show that ABCA1 expression is required for promotion of cholesterol efflux during acute incubations, even when apoA-I has already acquired phospholipids by the ABCA1 pathway.

Overexpression of ABCA1 in BHK cells increased transport of cholesterol to sites accessible to exogenously

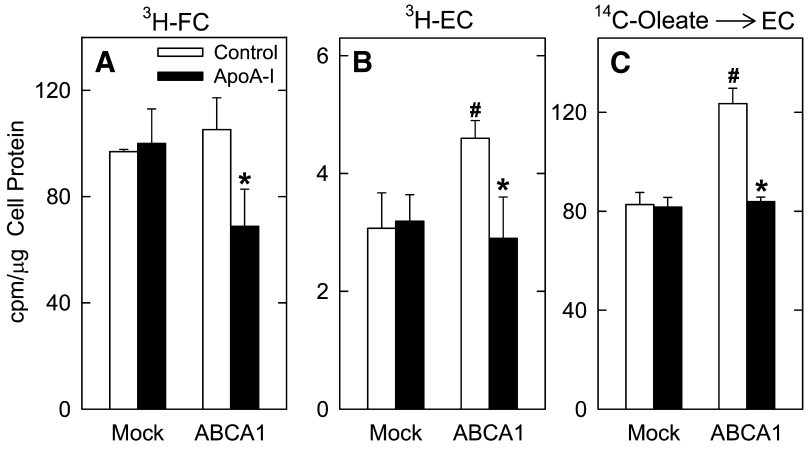

Fig. 5. ABCA1-promoted cholesterol esterification. A, B: Mifepristone-treated cells were radiolabeled with [^{3}H]cholesterol for 1 h and incubated without (Control) or with 10 μg/ml apoA-I for 6 h, and free (FC) and esterified (EC) cholesterol were measured. Results are the mean ± SD of mean values from four experiments, each performed in triplicate, expressed as cpm/μg cell protein. C: Cells were incubated for 6 h with 10 nM mifepristone plus 18 uM [^{14}C]oleate bound to albumin without (Control) or with apoA-I, and cellular cholesteryl [^{14}C]oleate and [^{14}C]triglycerides (not shown) were measured. Results are the mean ± SD of triplicates. #$P < 0.01$ versus mock control. *$P < 0.05$ versus ABCA1 control.

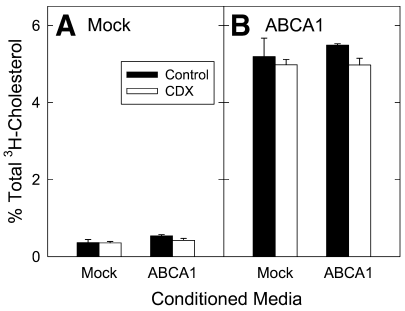

Fig. 6. Dependence on ABCA1 expression for apoA-I-stimulated cholesterol efflux. Medium containing 10 μg/ml apoA-I was incubated for 4 h with mifepristone-treated mock or ABCA1 transfectants or the same cells treated with methyl-β-cyclodextrin (CDX), the conditioned media were transferred to [^{3}H]cholesterol-labeled, mifepristone-treated mock (A) or ABCA1 (B) transfectants, and [^{3}H]cholesterol efflux was measured after 2 h incubations. Results are the mean ± SD of triplicates.

added cholesterol oxidase. Addition of apoA-I promoted cholesterol efflux exclusively from these sites, indicating that they were the source of cholesterol removed by apolipoproteins. In contrast, phospholipid-rich HDL particles, which remove cellular lipids by ABCA1-independent diffusion mechanisms (2), promoted cholesterol efflux from sites distinct from those accessible to oxidase. Because this ABCA1-generated pool of oxidase- and apolipoprotein-accessible cholesterol was detected when cells were treated with oxidase at 15°C, a temperature that inhibits enzyme internalization and cholesterol trafficking (25, 29), it was likely to be on the cell surface. These findings fit the hypothesis that apoA-I removes cholesterol that accumulates on the surface of the plasma membrane in response to increased expression of ABCA1 (21, 31).

Without ABCA1 expression, 10% or less of the radiolabeled cholesterol was accessible to cholesterol oxidase in BHK cells, similar to what has been described for other cell types (26, 32). ABCA1 induction increased the oxidase-accessible fraction to 20–30%, a 2- to 4-fold increase. Previous results showed that cholesterol loading of fibroblasts increased the fraction of oxidase-accessible cholesterol from ~10% to 30% (26). It now appears likely that cholesterol-induced ABCA1 was at least partially responsible for this increase. This conclusion is supported by the finding that oxidase-accessible cholesterol was below normal in cholesterol-loaded Tangier disease fibroblasts having dysfunctional ABCA1 (26).

We have yet to characterize the physical properties of the cholesterol oxidase-accessible membrane domains in these ABCA1-expressing BHK cells. Previous studies with cholesterol-loaded cells, however, showed that apoA-I removed cholesterol from membrane domains that were distinct from sphingolipid-rich rafts (26), were enriched with cholesterol and phosphatidylcholine (31, 33), and had a novel protruding morphology (21). It remains to be determined whether the oxidase-accessible cholesterol generated in response to forced overexpression of ABCA1 has the same properties.

Induction of ABCA1 in BHK cells also increased flux of cholesterol into the ACAT substrate pool, which was prevented in the presence of apoA-I. Apparently, when not removed by apolipoproteins, a fraction of the ABCA1-transported cholesterol traffics from the cell surface to intracellular sites, where it is esterified. These results raise the possibility that the previously described inward flux of plasma membrane cholesterol to ACAT (34, 35) is at least partially mediated by ABCA1.

Several possible mechanisms could account for the ABCA1-mediated cholesterol redistribution. ABCA1 could flop cholesterol and phospholipids from the inner leaflet of the plasma membrane to cell-surface domains that, when not solubilized by apolipoproteins, are endocytosed into intracellular compartments (1). In support of this idea are studies showing that another ABC transporter, P-glycoprotein, has both phospholipid and cholesterol floppase activities (36, 37). Alternatively, vesicles containing ABCA1 and perhaps apoA-I could recycle between the plasma membrane and intracellular compartments, transporting lipids along with them. Consistent with this process are studies showing that ABCA1-containing vesicles rapidly recycle between the plasma membrane and late endosomes (38), that apoA-I undergoes retroendocytosis in macrophages (39, 40), and that cholesterol is transported from the Golgi to the plasma membrane during apoA-I-mediated lipid efflux (41). Our data, however, do not exclude the possibility that ABCA1 alters the properties of the plasma membrane so as to make more cholesterol in the exofacial leaflet available for enzyme interactions. Whatever the mechanism, it is likely that the redistribution to these domains is physiologically relevant, as this cholesterol pool is the same one targeted for removal by apoA-I.

The current cholesterol redistribution data are inconsistent with the idea that ABCA1-mediated cholesterol efflux is a secondary response to forming phospholipid-rich acceptors. Moreover, incubating ABCA1-expressing cells with apoA-I for 4 h did not increase its ability to remove cholesterol from cells lacking ABCA1 during subsequent 2 h incubations. Thus, transporting phospholipids to apoA-I by the ABCA1 pathway followed by cholesterol diffusion could not account for the high level of cholesterol efflux from ABCA1-expressing cells. These findings differ from those reported by Wang et al. (19), who showed a substantial increase in the ability of apoA-I to promote cholesterol efflux after being transferred from ABCA1-transfected HEK293 cells to ABCA1-deficient cells. Possible explanations for these differences are that, compared with our BHK cells, the HEK293 cells may have had a higher fraction of diffusible radiolabeled cholesterol or a greater ability to generate phospholipid-rich apoA-I. It would be expected that ABCA1 would eventually transport enough phospholipids to apoA-I to produce acceptors for cholesterol that diffuses from cells.

This study does not exclude the possibility that ABCA1 transports cholesterol through its interactions with phospholipids. ABCA1 promotes phospholipid transport even from cholesterol-depleted cells (19, 42). Cross-linking experiments with photoactivated lipids suggested that ABCA1 interacts directly with phospholipids but not with

cholesterol (19). It is possible that ABCA1 cotransports phospholipids, cholesterol, and other molecules that interact with phospholipids. In support of this idea are results showing that apoA-I also removes α-tocopherol from cells by the ABCA1 pathway (43), indicating a broad specificity for lipophilic substrates. Because ABCA1 is induced by sterols (44, 45), its activity would only be evident in cells having high cholesterol-phospholipid ratios in membranes, making cholesterol the most physiologically relevant phospholipid partner transported by ABCA1.

In summary, the current study shows that inducing ABCA1 redistributes cholesterol to cell-surface sites, where it becomes available for removal by apolipoproteins. These findings support the apolipoprotein microsolubilization model of lipid efflux (20, 21), whereby apolipoproteins simultaneously remove phospholipids and cholesterol from cell-surface domains formed by ABCA1. Because ABCA1 also appears to mediate efflux of α-tocopherol by the same mechanism, it is likely that this simultaneous transport involves other molecules that interact with phospholipids.

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