Studies show that lipid-free apoA-I stimulates release of cholesterol and phospholipid from fibroblasts and macrophages. ATP-binding cassette 1 (ABC1) is implicated in this release and has been identified as the genetic defect in Tangier disease, evidence that ABC1 is critical to the biogenesis of high density lipoprotein. We quantified levels of ABC1 mRNA, protein, and cholesterol efflux from J774 mouse macrophages ± exposure to a cAMP analog. Up-regulating ABC1 mRNA correlated to increased cholesterol efflux in a dose- and time-dependent manner. mRNA levels rose after 15 min of exposure while protein levels rose after 1 h, with increased efflux 2–4 h post-treatment. In contrast to cells from wild-type mice, peritoneal macrophages from the ABC1–/– mouse showed a lower level of basal efflux and no increase with cAMP treatment. The stimulation of efflux exhibits specificity for apoA-I, high density lipoprotein, and other apolipoproteins as cholesterol acceptors, but not for small unilamellar vesicles, bile acid micelles, or cyclo-dextrin. We have studied a number of cell types and found that while other cell lines express ABC1 constitutively, only J774 and elicited mouse macrophages show a substantial increase of mRNA and efflux with cAMP treatment. ApoA-I-stimulated efflux was detected from the majority of cell lines examined, independent of treatment.

Reverse cholesterol transport is the process by which cholesterol in peripheral tissues is redistributed to sites of excretion and metabolism such as the liver and endocrine tissues (1, 2). Reverse cholesterol transport is thought to be mediated by high density lipoprotein (HDL). The initial step in this process is the release of free cholesterol and phospholipid from the plasma membrane of cells to acceptor particles (2–4). Efflux of free cholesterol occurs by a number of mechanisms, including aqueous diffusion to a phospholipid-containing acceptor (4), efflux to HDL mediated by receptors such as SR-B1 (5, 6), as well as release of membrane phospholipid and cholesterol to apolipoproteins (7–10). There is evidence that this latter process involves the interaction of apolipoprotein with a specific receptor(s) on the surface of certain cell types (10–12).

Support for the role of a binding event comes from studies indicating inhibition of efflux by treatment of cells with trypsin (11, 12), probucol (12, 13), monensin, and brefeldin A (14), as well as sulfobromophthalein and 4,4-diisothiocyanostilbene-2,2′-disulfonic acid (15). In addition, binding and lipid efflux to apolipoproteins has been stimulated by treatment of murine macrophages with cAMP analogs (11, 12, 16). Also, a variety of cells show enhanced phospholipid and cholesterol efflux to apoA-I upon enrichment with free cholesterol (17–20). ApoA-I has been directly cross-linked to cAMP-induced receptors (12), and co-eluted with cubulin, the intrinsic factor vitamin B12 receptor (21).

Defects in apoA-I and HDL metabolism characterize the inherited HDL deficiency, Tangier disease. Skin fibroblasts from patients with Tangier disease exhibit efflux to general acceptors of cholesterol (22) but lack specific efflux to apoA-I (23–26), thus implying the involvement of a receptor for apoA-I. Patients have low levels of HDL and apoA-I, presumably due to the high turnover of under-lipidated particles (27). This leads to striking pathology characterized by increased stores of cholesteryl ester and phospholipid in many cell types. Recent publications have revealed the underlying genetic defect as mutation of the ATP-binding cassette 1 (ABC1) (15, 28–31). ABC1 appears to be a pore-forming protein consisting of 6–6 transmembrane domains connected by a hydrophobic segment (32). It has two nucleotide-binding domains, which bind ATP and hydrolyze it in order to power active transport of unknown substrate(s). It was first identified in macrophages as a potential transporter for interleukin-1 (32), however, it is unclear if it plays a role in inflammation. ABC1 is highly expressed in liver, adrenals, small intestine, fetal tissues, placenta, and brain where it may affect development through apoptosis and/or phagocytosis (32). ABC transporters have been associated with many diseases such as drug-resistant cancer (33, 34), diabetes (35, 36), cystic fibrosis, and cholestasis (37), among others, making these proteins ideal targets for therapeutic intervention (38).
undertaken to determine if increased efflux of cholesterol was correlated to the up-regulation of ABC1 protein and mRNA. Using J774 as a model system, we established a standard protocol, which we then applied to a variety of cell types. This revealed a close correlation between ABC1 message levels and increased efflux. The relationship was confirmed by studies of elicited macrophages from the Abc1−/- mouse.

**EXPERIMENTAL PROCEDURES**

### Materials

The following were purchased from Sigma: phospholipid 12-myristate 13-acetate, fetal bovine serum (FBS), calf serum, bovine serum albumin (essentially fatty acid free), gentamicin (g), iodoacetamide, 2-deoxyguanosine, 1,2-[3H]cholesterol, and 1-(1-benzyl-1H-tetrazol-5-yl) 3-(4-dimethylaminophenyl)-5-(3-carboxymethoxyphenyl)-9H-xanthene sulfonic acid (XAMSA). 2-Hydroxypropyl-β-cyclodextrin (2- OH-β-CD) was a gift from Cerestar USA, Inc. (Hammond, IN). Organic solvents were purchased from Fisher Scientific (Pittsburgh, PA). [1,2-3H]Cholesterol (specific activity = 45 Ci/mmol) was from NEN Life Science Products Inc. Tissue culture flasks and plates were from Corn ing (Corning, NY) and Falcon (Lincoln, NJ). Minimum essential medium buffered with 25 mM Hepes, pH 7.4 (MEM-Meps), Dulbecco’s minimum essential medium (DMEM), RPMI 1640, Eagle’s minimum essential medium (EMEM), phosphate-buffered saline and sodium pyruvate were purchased from CelLogro (Herndon, VA), and Ham’s F-12 was purchased from BioWhitaker (Walkersville, MD). Pefabloc and essential medium (EMEM), phosphate-buffered saline and sodium pyruvate were purchased from Roche Molecular Biochemicals (Indian apolis, IN). The acyl-CoA:acyltransferase (ACAT) inhibitor, Pfizer CP-113, 818, was a gift from Pfizer Inc. (Grotto, CT).

### Cell Culture—J774 Mouse macrophages were grown in RPMI 1640 with 10% FBS. U-937 human monocytes were grown in suspension in RPMI 1640 supplemented with 10% FBS, 0.1% sodium pyruvate, 0.25% glucose. THP-1 human monocytes were grown in the same medium supplemented with 50 µg/ml mercaptoethanol. THP-1 and U-937 were differentiated before use by incubating them for 3 days on growth medium supplemented with 50 ng/ml phorbol 12-myristate 13-acetate. COS-7 primate kidney cells were grown on DMEM with 10% calf serum. CaCo2, human enterocytes, were grown in DMEM with 20% FBS and 2 mM glutamine. Chinese hamster ovary cells (CHO-K1) were grown in Ham’s F-12 with 5% FBS. Fu5AH rat hepatoma cells, HepG2 human hepatoma cells, L-cell mouse fibroblasts and WI38/VA13 cells (SV40-transformed human lung fibroblasts) were grown in EMEM with 10% FBS for HepG2 and fibroblasts and 5% calf serum. CaCo2, human enterocytes, were grown in DMEM with 20% FBS, 0.25% glucose. THP-1 human monocytes were grown in the same medium supplemented with 50 µg/ml mercaptoethanol. THP-1 and U-937 were differentiated before use by incubating them for 3 days on growth medium supplemented with 50 ng/ml phorbol 12-myristate 13-acetate. COS-7 primate kidney cells were grown on DMEM with 10% calf serum. CaCo2, human enterocytes, were grown in DMEM with 20% FBS and 2 mM glutamine. Chinese hamster ovary cells (CHO-K1) were grown in Ham’s F-12 with 5% FBS. Fu5AH rat hepatoma cells, HepG2 human hepatoma cells, L-cell mouse fibroblasts and WI38/VA13 cells (SV40-transformed human lung fibroblasts) were grown in EMEM with 10% FBS for HepG2 and fibroblasts and 5% calf serum for Fu5AH. GM3468A normal human skin fibroblasts were grown in EMEM with 20% FBS. MFMs were isolated from male B6C3F1 mice 5 days after GM3468A normal human skin fibroblasts were grown in EMEM with 10% FBS for HepG2 and fibroblasts and 5% calf serum. DMEM-Hepes, Dulbecco’s modified essential medium (EMEM), phosphate-buffered saline and sodium pyruvate were purchased from Cellogro (Herndon, VA), and Ham’s F-12 was purchased from BioWhitaker (Walkersville, MD). Myristate 13-acetate, fetal bovine serum (FBS), calf serum, bovine serum albumin (essentially fatty acid free), gentamicin (g), iodoacetamide, 2-deoxyguanosine, 1,2-[3H]cholesterol, and 1-(1-benzyl-1H-tetrazol-5-yl) 3-(4-dimethylaminophenyl)-5-(3-carboxymethoxyphenyl)-9H-xanthene sulfonic acid (XAMSA) were purchased from Roche Molecular Biochemicals (Indianapolis, IN). The acyl-CoA:acyltransferase (ACAT) inhibitor, Pfizer CP-113, 818, was a gift from Pfizer Inc. (Grotto, CT).

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### Protein Isolation and Western Blotting Analysis—Total RNA was isolated by the single step method using TriZol.R. After extraction, RNA was precipitated with isopropyl alcohol and its integrity was assessed by agarose gel electrophoresis. Fifteen micrograms of total RNA were separated on a 1% agarose gel containing 2.2 mM formaldehyde. RNA was transferred to Hybond-XL nylon membranes (Amersham Pharmacia Biotech) and cross-linked. A 518-base pair probe extended from positions 3110 to 3627 on the murine ABC1 cDNA was used to detect expression with the rapid hybridization system (Amersham Pharmacia Biotech) according to the manufacturer’s conditions. To verify equal loading of RNA, ABC1 probes were stripped and the membrane was reblotted with a mouse cyclophilin probe (Ambion, Austin, TX). 32P radioactive emissions were recorded on a phosphor screen and scanned with a PhosphorImager (Fuji, Stamford, CT) to detect over a 5-order linear range of sensitivity. The hybridization signals were digitized and quantified.

### Statistical Significance—Results are reported as mean ± S.D. Statistical significance was determined by two-tailed Student’s t test (GraphPad Prism version 3.0, GraphPad Software, San Diego, CA).

### RESULTS

**Cholesterol Efflux from J774 Macrophages to Different Acceptors**—Our initial objective was to study the effect of cpt-cAMP treatment on cholesterol efflux and ABC1 mRNA in the J774 mouse macrophage system. Cell monolayers were prelabeled with 1,2-[3H]cholesterol together with an ACAT inhibitor to ensure that all of the radiolabeled cholesterol released from the cell was derived from an unesterified cholesterol pool. A standard protocol was adopted in which monolayers were treated for 12 h with cpt-cAMP. This was followed by a 4-h incubation with various acceptors, during which time the fractional release of cellular label was quantified (see Experimental Procedures). The absolute amount of efflux differed among the acceptors, a reflection of differences in concentration and efficiency. Cpt-cAMP treatment resulted in a marked stimulation of cholesterol efflux to apoa-A-I (14-fold) and HDL (3-fold) (Fig. 1). In contrast to apolipoprotein-containing acceptors, efflux to other acceptors (2-OH-β-CD, SUVs, bile acid micelles) was not increased by cpt-cAMP treatment (Fig. 1).
Furthermore, cpt-cAMP enhanced efflux to other exchangeable apolipoproteins as shown in Fig. 2. At equivalent mass concentrations, efflux was greatest to apoA-I and lowest to apoC-III. Thus, cpt-cAMP-stimulated efflux can be observed for exchangeable apolipoproteins and HDL, but not for other acceptors of cholesterol which have no protein component, such as CD, SUVs, or bile acid micelles.

In another series of experiments performed under the same protocol, 0.3 mM 8-Br-cGMP (8-bromoguanosine 3′,5′-cyclic monophosphate) was substituted for cpt-cAMP. No stimulation of efflux was observed (data not shown). However, when J774 cells were pretreated with prostaglandin E₁ or E₂ (0.1 mM), cholesterol efflux was stimulated 7- and 9-fold, respectively (data not shown).

Up-regulation of ABC1 by Cpt-cAMP—To determine if the increased efflux in response to cpt-cAMP was a result of increased ABC1, J774 cells were pretreated with cpt-cAMP over a range of concentrations and the relationship between ABC1 mRNA and efflux was examined. After 12 h of exposure to cpt-cAMP, there was a dramatic increase in ABC1 mRNA expression (Fig. 3).

Time Course of Cpt-cAMP Up-regulation of ABC1 mRNA, Protein, and Efflux—Stimulated efflux also depends on the duration of treatment with cpt-cAMP (12). A time course of incubation was performed to determine the kinetics of ABC1 mRNA expression, protein synthesis, and efflux (Fig. 4). An increase in ABC1 message was detected within the first 15 min of treatment (1.2-fold over time zero). Cpt-cAMP elicited a reproducible biphasic increase in ABC1 mRNA levels (Fig. 4). An initial peak of induction was observed at 2 h. At later times, a second phase of ABC1 mRNA induction was observed which peaked at 6 h (15-fold above levels at time 0) and was much more prolonged than the initial transient increase. Protein levels begin to rise steadily after the first hour of treatment, while increased efflux is detected after 2 h (Fig. 4). From this and other data, the rate of change in efflux is greatest between 6 and 8 h after treatment (Fig. 4). Thus, a rise in ABC1 message and protein preceded efflux and there was a parallel increase in protein and efflux thereafter. Interestingly, two bands of ABC1 protein were detected (Fig. 5); the lower molecular mass species migrated at approximately 210 kDa while the higher molecular mass species was also induced by cpt-cAMP and may have been a phosphorylated or post-translationally processed form of ABC1 protein.

Cpt-cAMP Stimulation of Efflux from Elicited Peritoneal Macrophages—Cells were incubated in the presence and absence of 0.5 mM cpt-cAMP for 12 h and then harvested. Total RNA was isolated and 30 μg were loaded per lane. After transfer, the blot was probed with murine ABC1 cDNA (A), stripped and probed with murine cyclophilin cDNA (B) as described under “Experimental Procedures.” kb, kilobase.
Cells were treated with 0.3 mM cpt-cAMP and protein was
phages. J774 cells were seeded at 5 x 10⁵/100-mm dish, grown for 2
days to confluence, incubated with ACAT inhibitor and equilibrated as
described under “Experimtnal Procedures.” At the beginning of the
experiment, a set of 100-mm plates was harvested for baseline ABC1
mRNA and protein and parallel monolayers were treated with 0.3 mM
cpt-cAMP. Monolayers were sequentially harvested over the course of
8 h. In a separate experiment with triplicate wells, a radiolabeled set
of cells was used to determine cholesterol efflux to lipid-free apoA-I (20
µg/ml) in the presence of 0.3 mM cpt-cAMP. (Fig. 7). The control
ABC1 was detected with a rabbit primary antibody to murine ABC1 and
Procedures.” Six micrograms of total protein were loaded per lane.
isolated as described in the legend to Fig. 1 and under “Experimental
initially developed to study the relationship of ABC1 to inter-
leukin-1 levels, is a valuable model of Tangier disease (41). Elicited peritoneal macrophages from DBA-1J wild-type and
knockout mice show different patterns of efflux to apoA-I (Fig.
6). In wild-type cells, efflux to lipid-free apoA-I was up-regu-
lated 2-fold by treatment with cpt-cAMP. No stimulation was
observed with macrophages from the Abc1 knockout. Also,
there was a 3-fold difference in the basal rate of efflux to free
apoA-I from wild-type macrophages compared with those from
the knockout (Fig. 6).

**Cpt-cAMP-stimulated Efflux from Various Cell Types—**Cholesterol efflux from murine macrophages (J774, MPM, and
RAW 264) to apoA-I is highly responsive to cAMP stimulation
(11, 12, 16). We extended our standard protocol of 12 h labeling,
followed by 4 h efflux to the analysis of a number of cell lines. J774
and MPM showed both up-regulated ABC1 message (in a
representative experiment (n = 3): 5.7- and 1.6-fold increases,
respectively, data not shown) and enhanced efflux to lipid-free
apoA-I (5.8- and 2.5-fold increases above control, respectively)
(Fig. 7A). Less dramatic efflux responses to cpt-cAMP were
observed in THP-1 and COS-7 cells. Cells also expressed varying
levels of endogenous ABC1 mRNA (Fig. 8). It should be
noted that the expression levels of ABC1 mRNA from primate and
rodent cells cannot be compared directly since different
probes were used to analyze each set. Relative to MPM, J774
expressed lower levels of ABC1 mRNA, which increased upon
cpt-cAMP treatment. CHO-K1 cells expressed higher levels and
showed no cpt-cAMP-stimulated efflux. HepG2 cells and L-cells
were not stimulated by cpt-cAMP. Transformed human skin
fibroblasts (WI38/VA13), COS-7, CaCo2, Fu5AH, and
GM3468A cells showed low endogenous expression of ABC1
mRNA and no stimulation by cpt-cAMP. A strong relationship
between apoA-I-stimulated cholesterol efflux and endogenous
ABC1 mRNA levels was not apparent among the different cells,
although a general relationship appeared to be present among
the group of rodent cells. Thus, the level of ABC1 message
cannot be used to predict the rate of cholesterol release to
lipid-free apoproteins, suggesting that factors beside the level
of ABC1 contribute to cellular lipid efflux.

Lipid-free apoA-I induced efflux from cell lines that did not
show stimulation by cpt-cAMP (Fig. 7B). In cpt-cAMP-treated
cells, apoA-I-stimulated efflux was highest for J774, MPM, and
CHO-K1 (14-, 4.3-, and 6.9-fold increases above cells incubated

**Macrophages from Control and Abc1 / / Mice—**The Abc1
 / / mouse model also provides strong evidence that a
cpt-cAMP-stimulated increase in cholesterol efflux to apoA-I is
mediated though the activation of ABC1. The Abc1 / / mouse,

![Graph depicting time course of changes in ABC1 mRNA, protein, and cholesterol efflux upon cpt-cAMP treatment of J774 macrophages.](image1)

![Graph showing time course of ABC1 protein expression in J774 macrophages.](image2)

![Graph illustrating cholesterol efflux from wild-type and Abc1 / / DBA-1J mice.](image3)
in the absence of apoprotein, respectively). These cells expressed high levels of ABC1 mRNA, either due to up-regulation (J774 and MPM), or endogenous expression (CHO-K1). The only cells that did not show apoA-I-stimulated efflux were CaCo2 and Fu5AH.

DISCUSSION

**ABC1 and Efflux**—ABC1 has recently been shown to play an important role in the formation of nascent HDL and in transporting cellular cholesterol and phospholipid to unassociated apoproteins or small HDL particles (15, 28–30). ABC transporters, as a class, handle a variety of substrates including peptides, ions, carbohydrates, vitamins, and lipids (for a review, see Ref. 49). While much is known about these transporters in general, very little is known about their higher order structure or function. The only member of the class for which there is structural data available is the P-glycoprotein, a flipase that catalyzes the movement of phosphatidylcholine from the inner leaflet of the plasma membrane to the outer leaflet (50–52). ABC1 itself shows greatest homology to the RIM protein which pumps vitamin A and lipid out of retinal rod cells (53), suggesting that it may have a similar mechanism of action in the transport of phospholipid and/or cholesterol out of cells.

Of the various cell types that have been shown to release lipid to apoproteins, mouse macrophages are among the most active. The apoprotein-mediated efflux of both cholesterol and phospholipid is dramatically increased if these cells are enriched with cholesterol or treated with cAMP analogs. Building on the discovery that ABC1 is of prime importance in HDL synthesis, we undertook the present investigation to determine if lipid efflux from J774 mouse macrophage cells is directly linked to the expression of ABC1. J774 is a good model system for studying the correlation between ABC1 levels and efflux since release to apolipoproteins from this cell line is markedly up-regulated by both cholesterol enrichment and cAMP treatment (12, 19).

**Factors Modulating ABC1 Expression**—A standard experi-
mental approach was used in this study in which cells were pretreated with cpt-cAMP and the release of radiolabeled cellular cholesterol to lipid-free human apoA-I was quantified. Efflux values obtained with cpt-cAMP pretreated cells were compared with those from untreated, control cultures. Differences in efflux between the two sets of cells reflected the up-regulation of the cholesterol efflux mechanism. Stimulated J774 macrophages show increased efflux to HDL, apoA-I, as well as to the exchangeable apolipoproteins apoE, C-I, C-II, and C-III (Figs. 1 and 2), consistent with previous reports (9, 11, 12, 40). Other studies have demonstrated that efflux to apoA-II and A-IV are also enhanced in cpt-cAMP-treated J774 (39). In contrast, no stimulation of efflux was observed when J774 cells were incubated with apoprotein-free acceptors such as 2-OH-β-CD, SUVs, or bile acid micelles (Fig. 1), indicating that an apoprotein-specific interaction is required. It should be noted that the ability of cpt-cAMP to stimulate cholesterol efflux from J774 cells to HDL is somewhat variable, with some experiments showing no increase in efflux above control (data not shown). This variability may be due to contamination of different HDL preparations with unassociated apoproteins and/or small pre-β-HDL particles. Studies by Fournier et al. (39) using mouse sera from animals transgenic for human apoA-IV show that the greatest stimulation of efflux from cpt-cAMP-treated J774 cells is obtained with the lipoprotein-free fraction containing apoproteins not associated with larger, less dense HDL particles. Gentle trypsinization of HDL reduces its ability to remove cholesterol (54). This could be consistent with a model in which ABC1 interacts with loosely associated apoproteins, a state that may render them more sensitive to trypsin.

One of the objectives of the present investigation was to determine if the cpt-cAMP-stimulation of efflux from J774 cells was a direct result of the up-regulation of ABC1 receptor. The results obtained from a number of different experiments convincingly demonstrate such a relationship. We first demonstrated that increasing concentrations of cAMP in the 12-h pretreatment phase of the experiment increases the level of ABC1 mRNA, and that the degree of enhancement of ABC1 mRNA correlates well with the observed increase in cell cholesterol efflux to apoA-I. A second, more detailed analysis of the time course of changes in ABC1 mRNA, ABC1 protein, and cell cholesterol efflux to apoA-I following addition of cAMP was also undertaken. A significant increase in ABC1 mRNA was detected as soon as 15 min after the exposure to cAMP, with measurable increases in both ABC1 protein and cholesterol efflux apparent by 2 h (Figs. 4 and 5). The stimulation of both receptor protein and efflux exhibited a parallel increase throughout the 8-h experimental period (Fig. 4). Interestingly, the change in ABC1 mRNA levels was biphasic, with a rapid and transient increase peaking at 2 h, followed by a decline and a second, higher, and more prolonged peak (Fig. 4). Similar changes have previously been reported for the fos and jun gene families as well as for other immediate early genes (55, 56).

The most convincing evidence directly linking ABC1 and apoA-I-mediated cholesterol efflux in macrophages was obtained by comparing elicited macrophages from control and Abc1 knockout mice. Cholesterol efflux from control macrophages was readily stimulated by cpt-cAMP, like that of J774 cells, while macrophages from Abc1 knockout mice showed no detectable increase in apoA-I-mediated efflux when treated with cpt-cAMP (Fig. 6). In addition, background levels of fractional efflux from the Abc1 knockout macrophages were markedly lower than those from control macrophages (Fig. 6).

Studying different modulators of efflux may provide clues as to which pathways are involved in up-regulating the ABC1 gene. Previous studies suggest that apoA-I and HDL induce efflux through intracellular signaling via phospholipases C and D, as well as protein kinase C (57, 58). In our study we examined the relationship of efflux to exogenous and endogenous levels of cAMP. J774 cells are stimulated by cpt-cAMP and 8-bromo-cAMP, two different analogs of cAMP. Cholesterol efflux is also stimulated by the elevation of intracellular cAMP levels with prostaglandins E1 and E2. These results suggest that G-protein-coupled prostaglandin receptors are involved in the cAMP-dependent stimulation of cholesterol efflux from J774 macrophages.

**ABC1 Expression in Other Cell Lines**—Cholesterol efflux from other cell types was studied in order to establish the relationship between ABC1 mRNA expression, cpt-cAMP responsiveness, and apoA-I-mediated efflux. Cell lines were screened under a standard set of experimental conditions (i.e., 0.3 mM cpt-cAMP/12 h, 4 h efflux to 20 μg/ml apoA-I). ABC1 mRNA was determined for pairs of control and cpt-cAMP-treated cells, and values were expressed relative to cyclophilin. Only J774 and elicited mouse peritoneal macrophages exhibited enhanced cholesterol efflux upon exposure to cpt-cAMP under these conditions (Fig. 7A). The murine macrophages exhibited the highest levels of ABC1 message, particularly after cpt-cAMP treatment. Recent studies by Lown et al. (15) have also demonstrated the relationship between ABC1 expression and cholesterol efflux using the RAW264.7 mouse macrophage line. ABC1 mRNA levels varied among the other cell lines that we examined. For example, Chinese hamster ovary cells (CHO-K1) expressed a high endogenous level of ABC1 mRNA (Fig. 8A). Cpt-cAMP may fail to increase cholesterol efflux to apoA-I in this and other cell lines if the endogenous level of the receptor represents maximal expression. GM3468A, a normal human skin fibroblast, showed very low background expression of ABC1 mRNA (5% of unstimulated J774 ABC1 mRNA expression) and no up-regulation of either message or efflux after 12 h of treatment (Fig. 7A). Since cpt-cAMP stimulates ABC1 mRNA expression within 15 min in J774 (Fig. 4), it was anticipated that increased expression in GM3468A would be achieved after extended treatment. The lack of expression was surprising in light of the report that 24 h incubation with 8-bromo-cAMP increases message in human skin fibroblasts (15). Up-regulation of ABC1 mRNA was only achieved when human skin fibroblasts were exposed to cAMP analogs for the extended time period of 24 h (data not shown). Therefore, cAMP stimulation of ABC1 message in fibroblasts may represent secondary changes in growth and/or cholesterol content induced in the cells by prolonged exposure.

The ability of lipid-free apoA-I to stimulate cell cholesterol efflux above that observed in the absence of the apoprotein varied among the different cell types (Fig. 7B). This generally correlated with the expression of ABC1, consistent with the data obtained for J774. However, apoA-I enhanced cholesterol efflux even from cells expressing little ABC1. Basal levels of apoA-I-mediated efflux may be due to a protein/lipid interaction between amphipathic α-helical segments of the apoprotein with plasma membrane lipid domains, as opposed to the protein/protein interaction with ABC1 (for a discussion, see Ref. 59).

The present study demonstrates that ABC1 is the major protein mediating the efflux of cellular cholesterol to lipid-free apoproteins from murine macrophages, and that it is up-regulated by increasing cellular cAMP levels. However, a rapid, cAMP-induced response appears to be largely confined to murine macrophages and is not seen with a number of other cell types. Additional studies are necessary to elucidate the second messenger pathways operating in the mouse macrophage systems, and to establish why these pathways are not responsive
Correlation of ABC1 mRNA Levels with Cholesterol Efflux

in many other cells. Based on previous studies (18–20), it can be anticipated that other metabolic manipulation, namely enrichment of cells with cholesterol, will exhibit a more general effect on the up-regulation of ABC1. We have obtained preliminary data demonstrating an increase in ABC1 mRNA in macrophages enriched with free cholesterol (data not shown), and cholesterol enrichment studies are currently being conducted.

When viewed in retrospect, the large body of work on apolipoprotein-mediated cholesterol efflux may be re-evaluated in the light of the discovery of ABC1. The ability of ABC1 to lipidate cholesterol-rich macrophages enriched with free cholesterol (data not shown), may shed new light on the mechanism of ABC1 function.

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The Correlation of ATP-binding Cassette 1 mRNA Levels with Cholesterol Efflux from Various Cell Lines
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