# ATP binding cassette transporter ABCA1 modulates the secretion of apolipoprotein E from human monocyte-derived macrophages

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ABSTRACT **Apolipoprotein E (apoE) produced by macrophages in the arterial wall protects against atherosclerosis, but the regulation of its secretion by these cells is poorly understood. Here we investigated the contribution of the adenosine triphosphate binding cassette transporters ABCA1 and ABC8 to the secretion of apoE from either primary human monocyte-derived macrophages (HMDM) or human THP1 macrophages. During incubations of up to 6 h, apoE secretion from both THP1 macrophages and HMDM was stimulated by 8-Br-cAMP, which activates ABCA1 expression. The putative ABCA1 inhibitor glyburide and antisense oligonucleotides directed against ABCA1 mRNA significantly reduced apoE secretion from THP1 macrophages and HMDM. Antisense oligonucleotides directed against ABC8 mRNA also inhibited apoE secretion, although this inhibition was less pronounced and consistent than in the case of ABCA1. ApoE secretion from HMDM of ABCA1-deficient patients with Tangier disease was also decreased. ApoE mRNA expression was not affected by inhibition of ABCA1 or ABC8 in normal HMDM or the lack of functional ABCA1 in HMDM from Tangier disease patients. Inhibition of ABCA1 in HMDM prevented the occurrence of anti-apoE-immunoreactive granular structures in the plasma membrane. We conclude that ABCA1 and, to a lesser extent, ABC8 both promote secretion of apoE from human macrophages.—von Eckardstein, A., Langer, C., Engel, T., Schaukal, I., Cignarella, A., Reinhardt, J., Lorkowski, S., Li, Z., Zhou, X., Cullen, P., Assmann, G. ATP binding cassette transporter ABCA1 modulates the secretion of apolipoprotein E from human monocyte-derived macrophages. *FASEB J.* 15, 1555–1561 (2001)**

*Key Words: ABCA1 · Tangier disease · apoE*

Apolipoprotein E (apoE) is synthesized predominantly by the liver and is a major protein component of chylomicron remnants, very low density lipoproteins, and some subpopulations of high density lipoproteins (HDL) (1, 2). It mediates the clearance of these lipoproteins and their remnants from the circulation (1, 2). The hypolipidemic and antiatherogenic effect of apoE is illustrated by the accumulation of lipoprotein remnants in plasma and the early onset of atherosclerosis in both humans and mice lacking apoE (3–5).

Macrophages also produce apoE, although to a much lesser extent than hepatocytes (2, 6). Without producing gross changes in plasma lipid levels, expression of a human apoE transgene in macrophages of apoE-deficient mice prevented atherosclerosis (7) whereas transplantation of apoE-deficient macrophage stem cells into wild-type mice (8) promoted atherosclerosis. Possible explanations for the anti-atherogenicity of macrophage-derived apoE include the ability of macrophage-derived apoE to promote cholesterol efflux from these and other cells (2, 6, 9, 10), to inhibit the proliferation and migration of smooth muscle cells (11) and to interfere with the expression of cellular adhesion molecules by endothelial cells (12).

The mechanism and regulation of apoE secretion from macrophages are little understood. Enrichment of cells with cholesterol and activation of protein kinase A with the cyclic adenosine monophosphate analog 8-Br-cAMP stimulate the secretion of apoE (13, 14). ApoE secretion is also stimulated in the presence of exogenous HDL and apoA-I (15, 16). Finally, apoE secretion from macrophages is inhibited by brefeldin A, which disrupts structure and function of the Golgi apparatus, the *trans*-Golgi network (TGN), endosomes (17), as well as interferon-γ (18).

Regulation of apoE secretion from macrophages shares several properties with the regulation of choles-

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terol efflux from these cells. Cholesterol efflux is stimulated by the presence of apoA-I, HDL, and 8-Br-cAMP (19, 20) and is inhibited by brefeldin A (21) and interferon-γ (22). An important regulator of cholesterol efflux is the adenosine trisphosphate (ATP) binding cassette transporter 1 (ABCA1) (23). ABCA1 has been suggested to serve either as a regulator of vesicular transport between the TGN and the plasma membrane (24) or as a channel protein or so-called floppase within the plasma membrane (24–26). ABCA1 deficiency in Tangier disease disrupts apoA-I-mediated cholesterol efflux from cells and leads to HDL deficiency and foam cell formation (23, 27). Cholesterol loading and cyclic AMP treatment up-regulate ABCA1 expression in macrophages (20, 28–30).

Because of the similarities in the regulation of apoE secretion, cholesterol efflux, and ABCA1 activity, we investigated whether ABCA1 controls apoE secretion from macrophages. We also investigated the role of human ABC8, which shows homologies to the white gene in *Drosophila* and that, like ABCA1, was shown to be regulated by cholesterol and to mediate cholesterol efflux (29).

## MATERIALS AND METHODS

### Isolation of lipoproteins

LDL (1.019<d<1.063 g/ml) was isolated from plasma of normolipidemic volunteers by sequential ultracentrifugation (31) and acetylated as described by Brown and colleagues (32).

### Cells

Human monocytes were obtained from healthy volunteers and from two Tangier patients by leukapheresis and elutriation (13). The Tangier patients have been described and were homozygous for the N890S mutation (formerly denominated N875S mutation) and for a frameshift mutation in the ABCA1 gene, respectively (33, 34). Since the apoE genotype affects apoE secretion from macrophages (13), we took care that all donors had the apoE3/3 phenotype. Purity of the monocytes was controlled by FACS analysis and amounted to >95%. $10^6$ monocytes per 35 mm dish or $10^7$ monocytes per 75 $cm^2$ flask were differentiated into macrophages by 12 days cultivation in RPMI 1640 (BioWhittaker, Verviers, Belgium) containing 10% autologous human serum (Biochrom, Berlin, Germany) and penicillin (100 units/ml)/streptomycin (0.1 mg/ml) (Sigma, Deisenhofen, Germany) as antibiotics. THP1 monocytes were purchased from American Type Culture Collection (Manassas, VA) and cultivated with RPMI 1640 containing 20% fetal calf serum (Biochrom, Berlin, Germany) and penicillin (100 units/ml)/streptomycin (0.1 mg/ml) (Sigma) as antibiotics. $10^6$ THP1 monocytes were seeded into 35 mm dishes and differentiated into macrophages by 96 h incubation with the phorbol ester phorbol 12-myristate 13-acetate (100 nM PMA, Sigma) and 50 nM β-mercaptoethanol. For some experiments, human monocyte-derived macrophages (HMDM) and THP1 macrophages were converted into foam cells by loading with 100 μg/ml acetylated LDL (acLDL) for 48 h (32).

### Antisense oligonucleotides

Phosphothionate antisense oligonucleotides were used for the inhibition of ABCA1 and ABC8. The antisense oligonucleotide directed against the ABCA1 mRNA was purchased from Biognostik (Göttingen, Germany) and had the sequence 5′-CATGTTGTTCATAGGGTGGGTAGCTC-3′ (35); the antisense oligonucleotide directed against the ABC8 mRNA was purchased from Metabion (Martinsried, Germany) and had the sequence 5′-TGCCGACCGAGAAAG-3′ (29). As control oligonucleotides, we used the reverse complements of the target sequences. No cross homologies were found in the GenBank database. The efficient cellular uptake of these oligonucleotides was controlled by fluorescence microscopy of macrophages that were incubated with the FITC-labeled homologues of the antisense oligonucleotides. The two nucleotides were used at a concentration of 4.4 nmol/l at which they were previously shown to inhibit cholesterol efflux from cells by ~50% (29, 35).

### Quantification of apoE in media and cells

HMDM or THP1 macrophages were cultivated for varying intervals of time in serum-free RPMI 1640 that was supplemented with potential modulators of ABCA1 or ABC8. As a potential stimulator, we used 0.3 μmol/l 8-Br-cAMP (Calbiochem, Bad Soden, Germany). As potential inhibitors, we used different concentrations of glyburide (Calbiochem, Bad Soden, Germany), 4,4-diisothiocyanostilbene-2,2′ disulfonic acid, or bromosulfthalein (both from Sigma). If dimethyl sulfoxide (DMSO) was needed as the solvent, we used RPMI 1640 with DMSO as controls. Antisense oligonucleotides and control oligonucleotides were added to the medium at a final concentration of 4.4 nmol/l. After incubation, media were collected for the quantification of apoE by ELISA (13, 36). Cells were harvested with a rubber policeman for the quantification of cellular protein by the Lowry method or for the measurement of cellular apoE by ELISA. The apoE ELISA was performed using a monoclonal anti-apoE antibody (Dunn, Asbach, Germany) as the capturing antibody and IgG from a lab-made rabbit anti-human apoE antiserum as the second antibody. The immunoreaction was visualized by serial incubation with biotinylated anti-rabbit IgG (DAKO, Hamburg, Germany), streptavidin horseradish peroxidase as the conjugate, and ortho-phenylenediamine as the substrate (Sigma).

### Detection of apoE mRNA by Northern blotting

Total RNA was purified from macrophages by using the RNeasy mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol and treated with DNase (MessageClean kit, GenHunter, Nashville, TN) to remove residual contaminations with DNA. Single–stranded cDNA was prepared from 5 μg RNA using the SuperScript™ RT-PCR kit (Life Technologies, Eggenstein, Germany), yielding 20 μl. PCR was performed in a 50 μl reaction containing 1 μl of cDNA, 2.5 units of HotStarTaq (Qiagen), the supplied reaction buffer (Qiagen), 200 μM of each dNTP, and 0.5 μM of each primer. Sequences of the primers are listed in **Table 1**. Amplification was performed with a DNA thermal cycler (Applied Biosystems, GeneAmp, PCR system 9700, Branchburg, NJ) under the following conditions: hot start at 94°C for 15 min, followed by 40 cycles (94°C for 45 s, 59°C for 90 s, 72°C for 1 min), and a final incubation at 72°C for 7 min. PCR products of the human apoE gene (bp 14–321 of GenBank Acc NM 000041) and GAPDH gene (bp 81–306 of GenBank Acc. NM 002046) were gel purified using a 2% agarose TAE gel and a gel extraction kit (Qiagen). Digoxigenin (DIG)

TABLE 1. *Sequences of PCR primers*

| | |
|---|---|
| ApoE sense | 5′-AAG GAC GTC CTT CCC CAG GAG-3′ |
| ApoE antisense | 3′-CTT CAT GGT CTC GTC CAT CAG C-5′ |
| GAPDH sense | 5′-GAA GGT GAA GGT CGG AGT C-3′ |
| GAPDH antisense | 3′-GAA GAT GGT GAT GGG ATT TC-5′ |

-labeled probes were generated using the DIG DNA labeling and Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany). Sensitivity of the probe was determined by dot blot analysis.

For Northern blot analysis, 5 μg of total RNA was separated on a denaturing formaldehyde 1% agarose gel. The RNA was transferred onto a positively charged nylon filter (Roche Molecular Biochemicals) by capillary transfer and hybridized with the above-described DIG-labeled DNA probes directed against human apoE and GAPDH gene according to the manufacturer's protocol (DIG DNA Labeling and Detection Kit, Roche Molecular Biochemicals). The hybridization signal was detected with an anti-digoxigenin alkaline phosphatase conjugate and CDP-Star by chemoluminescence using Hyperfilm ECL detection (Amersham Pharmacia Biotech, Buckinghamshire, UK). Autoradiographs were scanned and processed with the AIDA program (Raytest, Straubenhardt, Germany.

### Immunofluorescence microscopy of apoE

Monocytes were seeded into 24-well cell culture dishes that contained coverslips with 12 mm diameter. After 14 days of cultivation and lipid loading as required, the cells were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized by incubation with 0.1% Triton X-100 in PBS. To suppress unspecific binding of antibodies, the cells were blocked with 20% heat-inactivated goat serum in PBS (Sigma). Thereafter, the cells were blocked and incubated with in-house rabbit anti-apoE antiserum and subsequently with Cy3-labeled goat anti-rabbit IgG (Dianova, Hamburg, Germany). Confocal immunofluorescence microscopy was performed with an Olympus Fluoview IX 70.

### Statistics

The paired *t* test was used to calculate levels of statistical significance.

## RESULTS

### Effects of 8-Br-cAMP and glyburide on apoE secretion

8-Br-cAMP increased apoE secretion from THP1 macrophages during incubations up to 6 h (acLDL-loaded cells, **Fig. 1***A*) or 3 h (non-loaded cells; not shown). Longer incubations with 8-Br-cAMP led to a significant decrease of apoE secretion (Fig. 1*A*). Likewise, 8-Br-cAMP increased apoE secretion from HMDM during incubations up to 6 h but decreased apoE secretion during incubations for 24 h (Fig. 1*B*).

Glyburide inhibited apoE secretion at concentrations >100 μmol/l. Maximal inhibition was reached at a glyburide concentration of 250 μmol/l and amounted to >90% and 80% in nonloaded and loaded THP1 macrophages, respectively (**Fig. 2**) and to 35% and 55% in unloaded and loaded HMDM, respectively (**Table 2**).

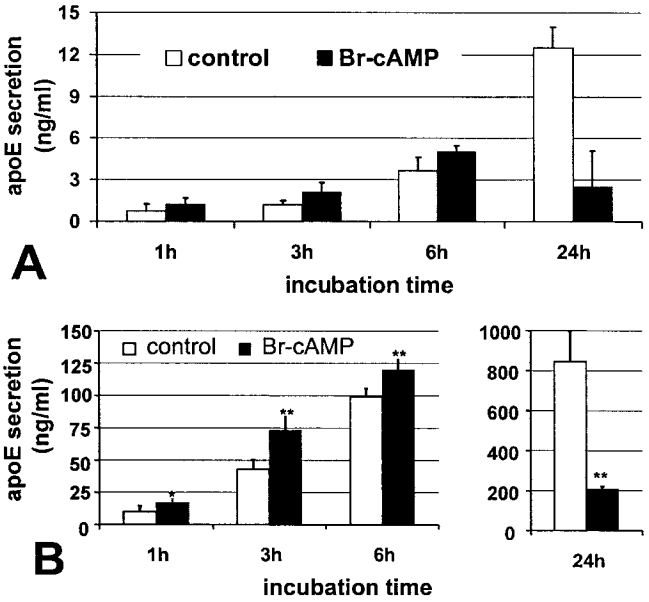


**Figure 1.** Effect of 8-Br-cAMP on apoE secretion from human macrophage foam cells. AcLDL-loaded THP1 macrophages (*A*) and HMDM (*B*) were incubated for the time intervals shown with 0.3 μmol/l 8-Br-cAMP. ApoE was measured in the medium by ELISA. $*P < 0.05$, $**P < 0.01$ (*t* test, triplicate experiment).

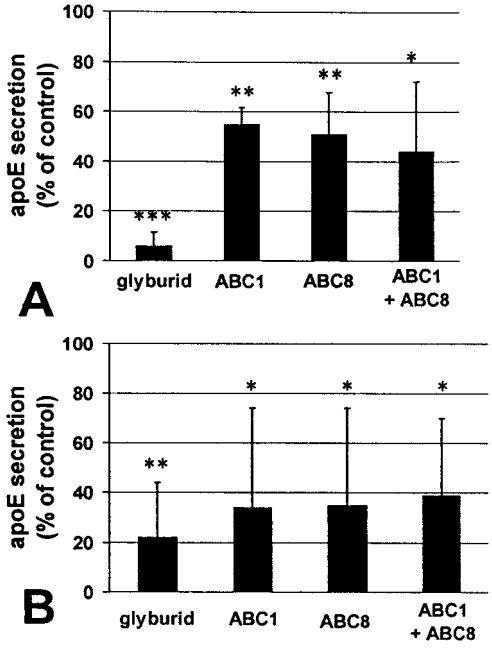


**Figure 2.** Effect of antisense oligonucleotides on apoE secretion from THP1 macrophages. Unloaded (*A*) or acLDL-loaded (*B*) THP1 macrophages were incubated for 8 h with 4.4 nmol/l antisense oligonucleotides directed against ABCA1 mRNA and/or ABC8 mRNA as well as (for positive control) with glyburide. $*P < 0.05$, $**P < 0.01$ (*t* test, $n$=3–5 experiments).

TABLE 2. *Effect of antisense oligonucleotides on apoE secretion from HMDM*[a]

| | ApoE secretion from nonloaded cells (ng/ml medium) | | | ApoE secretion from acLDL loaded cells (ng/ml medium) | | |
|---|---|---|---|---|---|---|
| | Control (sense) | Inhibitor (antisense) | *P* | Control (sense) | Inhibitor (antisense) | *P* |
| Glyburide | 183.8 ± 47.7 | 120.0 ± 15.5 | <0.05 | 177.7 ± 15.1 | 74.7 ± 2.89 | <0.01 |
| ABCA1 | 98.6 ± 2.5 | 19.2 ± 0.3 | <0.01 | 92.4 ± 7.6 | 50.9 ± 15.1 | <0.01 |
| ABC8 | 150.9 ± 8.9 | 117.2 ± 4.8 | <0.05 | 156.9 ± 66.5 | 157.7 ± 8.6 | n.s. |
| ABCA1 + ABC8 | 74.6 ± 2.9 | 17.7 ± 2.1 | <0.05 | 49.2 ± 8.1 | 12.0 ± 7.0 | <0.01 |

[a] HMDM macrophages were incubated for 8 h in the presence (inhibitor) or absence (control) of 250 μmol/l glyburide or with 4.4 nmol sense (control) or antisense oligonucleotides (inhibitor) against ABCA1 and/or ABC8. Data are from representative triplicate experiments. *P* was calculated by paired *t* test.

### ApoE secretion is decreased by antisense oligonucleotides directed against ABCA1 mRNA and in ABCA1-deficient cells

Compared with control oligonucleotides, antisense oligonucleotides directed against either ABCA1 or ABC8 mRNA reduced apoE secretion from nonloaded THP1 macrophages by 45% to 50% ($P<0.01$, Fig. 2*A*) and from acLDL-loaded cells by 65% to 70% ($P<0.01$, Fig. 2*B*). The combination of the two antisense oligonucleotides did not enhance the inhibitory effect of the single antisense oligonucleotides. It is noteworthy that the antisense oligonucleotides were less efficient in inhibiting apoE secretion from THP1 cells as compared to glyburide.

The antisense oligonucleotide directed against ABCA1 mRNA significantly decreased the secretion of apoE from nonloaded and acLDL-loaded HMDM by 80% and 45%, respectively ($P<0.01$; Table 2). The antisense oligonucleotide was as effective as glyburide in inhibiting apoE secretion from HMDM. The antisense oligonucleotide directed against ABC8 mRNA inhibited apoE secretion from unloaded HMDM by only 20% ($P<0.05$) and was ineffective at inhibiting apoE secretion from acLDL-loaded HMDM. The combination of anti-ABCA1 and anti-ABC8 antisense oligonucleotide did not increase the inhibitory effect of the anti-ABCA1-antisense oligonucleotides alone.

ApoE secretion from macrophages of two patients with Tangier disease was compared with apoE secretion from macrophages of two control donors that were cultivated in parallel with the Tangier cells and with apoE secretion from macrophages obtained from eight separate donations (**Fig. 3**). All healthy donors and Tangier patients exhibited the apoE3/3 phenotype. The apoE level in the media of Tangier macrophages was the lowest of all 12 macrophage cell isolates and amounted to ~50% of the mean apoE level in the media of control macrophages ($P<0.05$).

### Effect of ABCA1 and ABC8 on apoE mRNA and cellular apoE

The influence of ABCA1 inhibition or deficiency on apoE production was assessed by the measurement of apoE mRNA and cellular apoE in HMDM. Neither glyburide, 8-Br-cAMP, nor antisense oligonucleotides directed against ABCA1 or ABC8 mRNA affected the abundance of apoE mRNA relative to that of the housekeeping gene GAPDH (**Fig. 4**). ApoE gene expression in Tangier macrophages was also not different from that in normal macrophages (not shown).

The cellular content of apoE was measured by ELISA of cell lysates and did not change upon incubation of HMDM or THP1 macrophages with either glyburide or antisense oligonucleotides directed either against ABCA1 or ABC8. However, inhibition of ABCA1 with antisense oligonucleotides affected the distribution of apoE in HMDM (**Fig. 5**). In nonloaded and loaded HMDM, which were either incubated with medium alone or with control oligonucleotides (Figs. 5*A, B*), anti-apoE immunoreactive material was localized in perinuclear compartments, representing the endoplasmic reticulum and/or the Golgi apparatus. The granular staining in the cell periphery resembles plasma membrane and strong vesicular localization (Figs. 5*A,*

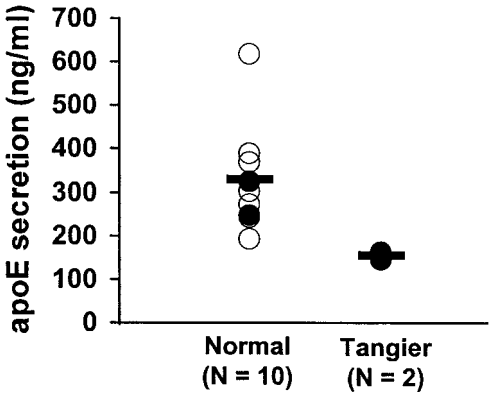


**Figure 3.** ApoE secretion is impaired in Tangier macrophages. AcLDL-loaded HMDM from two Tangier patients and 10 healthy controls were incubated for 24 h. Medium was harvested for quantification of apoE. Tangier cells secreted less apoE than macrophages from two control donors investigated in parallel (filled circles) and less than HMDM from eight donors that were investigated in separate experiments (open circles). The mean value of the two Tangier macrophage cell lines (154+13 ng/ml) was significantly different from the mean value of the 10 control HMDM cell lines (328+126 ng/ml) ($P<0.05$, *t* test).

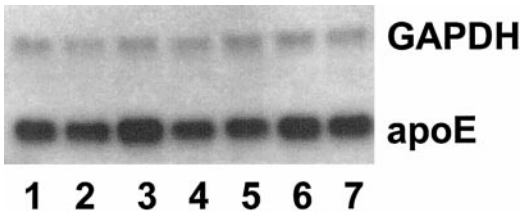


**Figure 4.** Effect of ABCA1 inhibition on the expression of apoE mRNA. AcLDL-loaded HMDM were incubated for 8 h with either medium alone (lane 1), 0.3 μmol/l 8-Br-cAMP (lane 2), 250 μmol/l glyburide (lane 3), 5 nmol/l anti-ABCA1 antisense oligonucleotide (lane 4), 4.4 nmol/l ABCA1 sense oligonucleotide (lane 5), 4.4 nmol/l anti ABC8 antisense oligonucleotide (lane 6), or 4.4 nmol/l ABC8 sense oligonucleotide (lane 7). RNA was isolated. Expression of apoE and GAPDH was measured by Northern blotting as described in Materials and Methods. Relative to GAPDH, no condition affected apoE gene expression.

*B*). Incubation of cells with anti-ABCA1-antisense oligonucleotides (Fig. 5*C*) led to the disappearance of this fine granular material in the periphery.

## DISCUSSION

We have provided several lines of evidence that ABCA1 and, to a lesser extent, ABC8 are involved in the secretion of apoE from both primary HMDM and transformed THP1 macrophages.

First, in agreement with the results of a previous study of apoE secretion from the murine macrophage cell line RAW264.7 (14), we found that apoE secretion increased from human macrophages in the presence of 8-Br-cAMP (Fig. 1). This activator of protein kinase A was previously shown to increase ABCA1 gene expression and ABCA1-mediated cholesterol efflux from macrophages (26, 30). However, in our study 8-Br-cAMP exerted stimulatory effects on apoE secretion during incubations up to 6 h, but had even more pronounced inhibitory effects during 24 h of incubation with macrophages. Since cyclic AMP is a very common second messenger that regulates a broad variety of metabolic processes (37), 8-Br-cAMP exerts contrasting effects on the cellular processing and secretion of apoE depending on the experimental conditions used. The stimulatory effect on apoE secretion possibly via ABCA1 activation (26, 30) appears to be rate-limiting only during short time incubation.

Second, apoE secretion was decreased by the sulfonylurea derivative glyburide (Fig. 2, Table 2), previously shown to inhibit ABCA1-mediated secretion of lipids, anions, and interleukin 1beta (IL-1β) as well as ABCA1-mediated engulfment of apoptotic cells by macrophages (35, 38–40). Glyburide was less efficient in inhibiting apoE secretion from HMDM than from THP1 cells.

Third, an antisense oligonucleotide directed against ABCA1 mRNA, previously shown to inhibit cholesterol efflux from fibroblasts by 50% (35), inhibited apoE secretion from both primary HMDM and transformed THP1 macrophages by at least 50% (Fig. 2, Table 2). An antisense oligonucleotide directed against ABC8 mRNA that had been shown to inhibit efflux of cholesterol and phospholipids from HMDM by 20 to 30% (30) inhibited apoE secretion, also by 50%, from THP1 cells and by 20% (Fig. 2) from unloaded HMDM. However, this antisense oligonucleotide was ineffective at inhibiting apoE secretion from acLDL-loaded HMDM (Table 2). The combination of the two antisense oligonucleotides did not increase the inhibitory effect of the anti-ABCA1 antisense oligonucleotide alone. These observations are similar to those made on the role of ABC8 in cholesterol efflux. Deficiency of ABCA1 completely abolishes cholesterol efflux from cells of patients with Tangier disease or ABCA1 knockout mice (23, 24, 26, 35, 40). Nevertheless, inhibition of ABC8 with an antisense oligonucleotide reduced cholesterol efflux, although less efficiently than an antisense oligonucleotide against ABCA1 (29, 35). ABCA1 and ABC8 hence may contribute to one transport mechanism where ABCA1 is the more active component.

Fourth, cholesterol-loaded macrophages from patients with Tangier disease have a reduced capacity to secrete apoE (Fig. 3). That functioning of ABCA1 is not a prerequisite for secretion of apoE from macrophages is illustrated by the fact that macrophages from patients with Tangier disease secrete about half as much apoE as control macrophages.

Taken together, the data presented here imply that ABCA1 and ABC8 contribute to the secretion of apoE by macrophages. We have ruled out that inhibition of

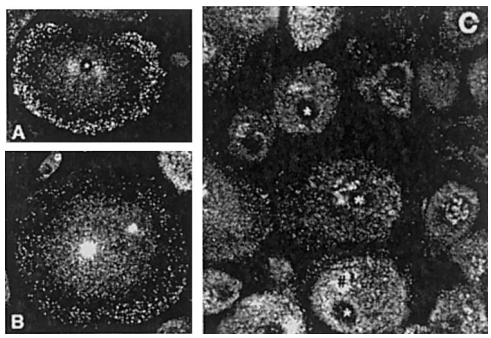


**Figure 5.** Effect of ABCA1 inhibition on the cellular distribution of apoE. Nonloaded HMDM were incubated for 8 h either in the absence of oligonucleotides (*A*) or in the presence of 4.4 nmol/l ABCA1 sense oligonucleotide (*B*) or anti ABCA1 antisense oligonucleotide (*C*). The fixed and permeabilized cells were incubated with a polyclonal rabbit anti-apoE antiserum and then Cy3-labeled goat anti-rabbit IgG. In unloaded cells incubated either without oligonucleotides or with control oligonucleotides, apoE was detected as diffuse material in the perinuclear region and as fine granular material in and beneath the cell membrane (*A, B*). Inhibition of ABCA1 led to the disappearance of this fine granular apoE-immunoreactive material (*C*). Symbols mark the nucleus (*) and possibly Golgi structures (#). Total cell height of the cells was 10–12 μm and the confocal sections (yx plane) represents sections at 4 μm.

ABC transporters A1 and 8 decreases apoE secretion. ApoE mRNA and cellular apoE levels were normal in macrophages that were incubated with antisense oligonucleotides and in macrophages of Tangier disease patients (Fig. 3). ABCA1 gene expression was even increased in the liver of ABCA1 knockout mice (41). It thus appears that inhibition of ABCA1 does not cause an increase in intracellular apoE levels, probably because excess intracellular apoE undergoes cellular degradation in either lysosomes or proteasomes (13, 17, 18, 42, 43).

Our data do not allow any conclusion as to the mechanism by which ABCA1 and ABC8 modulate apoE secretion. Net apoE secretion from macrophages is regulated in a complex manner (17). After production in the ER, a pool of nonglycosylated apoE is transported to the plasma membrane, where it is sequestered by binding to proteoglycans and apoE receptors. A considerable proportion is reinternalized and directed either to lysosomes for intracellular degradation or to the Golgi apparatus for glycosylation with sialic acid. This pool of sialo-apoE is transported to the cell surface for eventual secretion (17). This pathway resembles the retroendocytosis of apoA-I and HDL (44–48) previously found to be defective in ABCA1-deficient macrophages (44). ABCA1 may be involved in this transport at various stages. Since ABCA1 was shown to interact with lipid-free apolipoproteins on the plasma membrane (25, 26), ABCA1 may operate at the plasma membrane by facilitating the efflux of asialo- and/or sialo-apoE or the reinternalization of asialo-apoE. Since ABCA1 is responsible for the vesicular transport of lipids between intracellular compartments and the plasma membrane (24, 45), it may regulate the transport of asialo-apoE from the ER to the plasma membrane and/or from the plasma membrane to the Golgi and/or the transport of the sialo-apoE from the TGN to the plasma membrane. In agreement with a role of ABCA1 in the intracellular trafficking of apoE, we observed that inhibition of ABCA1 leads to the disappearance of apoE containing granular structures from the plasma membrane (Fig. 5). Likewise, ABCA1 was previously postulated to regulate the intracellular trafficking of the leaderless protein IL-1β from the Golgi apparatus to the plasma membrane for secretion (40, 48). In contrast to other apolipoproteins, which are synthesized as preproapolipoproteins, apoE is synthesized as preapoE. After the removal of the prepeptide in the endoplasmic reticulum, apoE does not contain a signal peptide (propeptide), so that like IL-1β, apoE may be considered a leaderless protein (49). ABCA1 hence may support apoE secretion by diverting the leaderless apoE from a degrading lysosomal or proteasomal pathway (13, 17, 18, 42, 43) to a secretory route.

In conclusion, we have demonstrated here that ABCA1 and to a lesser extent ABC8 modulate the net secretion of apoE from macrophages. In view of several anti-atherogenic properties of macrophage-derived apoE, our findings add a novel potentially anti-atherogenic property to ABC transporters A1 and 8. FJ

This work is part of a project supported by a grant from the Deutsche Forschungsgemeinschaft to A.v.E. (Ec116,3–6). A.v.E., S.L., A.C., and P.C. are participants in a 5th framework project entitled 'Macrophage and Plaque Stability' (QLG1–1999-01007) that is supported by the European Union.

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*Received for publication March 28, 2001.*
*Accepted for publication April 2, 2001.*