

**ATP-binding Cassette Transporter A1 Mediates Cellular Secretion of α-Tocopherol**

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α-Tocopherol (α-TOH) is associated with plasma lipoproteins and accumulates in cell membranes throughout the body, suggesting that lipoproteins play a role in transporting α-TOH between tissues. Here we show that secretion of α-TOH from cultured cells is mediated in part by ABCA1, an ATP-binding cassette protein that transports cellular cholesterol and phospholipids to lipid-poor high density lipoprotein (HDL) apolipoproteins such as apoA-I. Treatment of human fibroblasts and murine RAW264 macrophages with cholesterol and/or 8-bromo-cyclic AMP, which induces ABCA1 expression, enhanced apoA-I-mediated α-TOH efflux. ApoA-I lacked the ability to remove α-TOH from Tangier disease fibroblasts that have a nonfunctional ABCA1. BHK cells that lack an active ABCA1 pathway markedly increased secretion of α-TOH to apoA-I when forced to express ABCA1. ABCA1 also mediated a fraction of the α-TOH efflux promoted by lipid-containing HDL particles, indicating that HDL promotes α-TOH efflux by both ABCA1-dependent and -independent processes. Exposing apoA-I to ABCA1-expressing cells did not enhance its ability to remove α-TOH from cells lacking ABCA1, consistent with this transporter participating directly in the translocation of α-TOH to apolipoproteins. These studies provide evidence that ABCA1 mediates secretion of cellular α-TOH into the HDL metabolic pathway, a process that may facilitate vitamin transport between tissues and influence lipid oxidation.

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**EXPERIMENTAL PROCEDURES**

Lipoproteins and ApoA-I—LDL and HDL₃ (herein referred to as HDL) were prepared by sequential ultracentrifugation in the density range of 1.019 to 1.063 and 1.25 to 1.21 g/ml, respectively, and HDL was depleted of apoE and apoB by heparin-agarose chromatography.
Fig. 1. Comparison of α-TOH and cholesterol efflux from normal (NL) and Tangier disease (TD) fibroblasts.

Cells were radiolabeled with either [3H]cholesterol or [14C]α-TOH and either maintained in growth medium (Control) or treated with cholesterol plus 8-Br-cAMP (cholel-cAMP) as described under “Experimental Procedures.” Cells were then incubated for 6 h with medium containing 1 mg/ml BSA alone or BSA plus 5 µg/ml apoA-I, and medium and cellular radiolabel was measured. Results are the mean ± S.D. of triplicate incubations expressed as the percent of total radiolabeled α-TOH or cholesterol (UC) released into the medium. ApoA-I-mediated flux is the mean ± S.D. of values for BSA + apoA-I after subtraction of the mean value for BSA.

RESULTS

Previous studies showed that lipid-free apoA-I removes α-TOH from cultured cells (11). To test if this involves ABCA1, we compared the ability of apoA-I to remove radiolabeled α-TOH and cholesterol from fibroblasts expressing different amounts of ABCA1. Treatment of immortalized human fibroblasts with cholesterol plus 8-Br-cAMP markedly induces ABCA1 expression and apoA-I-mediated lipid efflux (16, 23). In contrast, fibroblasts from patients with Tangier disease have a nonfunctional ABCA1 whether or not they are treated with cholesterol/8-Br-cAMP (16, 23). When [14C]α-TOH- or [3H]cholesterol-labeled normal and Tangier disease fibroblasts were incubated for 6 h with BSA alone, a higher fraction of α-TOH than cholesterol appeared in the medium (Fig. 1), indicating that α-TOH diffuses from cells at a faster rate than cholesterol does. Addition of apoA-I to BSA-containing medium caused only a small or negligible increase in both α-TOH and cholesterol efflux from untreated (control) normal fibroblasts (Fig. 1, A and C) and from both control and cholesterol/8-Br-cAMP-treated Tangier disease fibroblasts (Fig. 1, B and D). In contrast, when ABCA1 was induced in normal cells by cholesterol/8-Br-cAMP, apoA-I-mediated efflux of both α-TOH and cholesterol increased 5-fold. Dose-response curves revealed that this induced efflux of α-TOH occurred by a high affinity ($K_d < 10^{-5} \text{ M}$) saturable process (Fig. 2), as is the case for ABCA1-mediated efflux of cholesterol and phospholipids (25). Thus, secretion of α-TOH from fibroblasts to apoA-I depends on an induced and functional ABCA1.

To examine further the role of ABCA1 in mediating α-TOH secretion, we compared the ability of different lipid acceptors to remove α-TOH from cholesterol/8-Br-cAMP-treated normal and Tangier disease fibroblasts. Lipid-free apolipoproteins remove cholesterol from cells by the ABCA1 pathway, whereas phospholipid-containing particles remove cholesterol by ABCA1-independent mechanisms (25–27). As in Fig. 1, addition of apoA-I to the medium increased α-TOH efflux from normal but not from Tangier disease fibroblasts (Fig. 3). The addition of HDL particles, which contain both phospholipids and apolipoproteins, increased α-TOH efflux from both normal and Tangier disease cells, but efflux was increased to a greater extent from normal cells. Trypsinized HDL, which lacks active apolipoproteins (21), also stimulated α-TOH efflux from cells, but there were no differences between normal and Tangier disease fibroblasts.
with [14C]normal and Tangier disease fibroblasts. Cells were radiolabeled with [14C]-α-Tocopherol, treated with cholesterol/8-Br-cAMP, and incubated for 6 h with medium containing 1 mg/ml BSA plus the indicated concentrations of apoA-I as described in the legend for Fig. 1. The results are the mean ± S.D. of triplicates expressed as percent of total radiolabeled α-Tocopherol released into the medium.

These results show that apoA-I removes an analog selectively induced apoA-I-mediated efflux of both compounds only in the presence of apoA-I, indicating that this cAMP analog selectively induces apoA-I-mediated efflux of both compounds. These results show that apoA-I removes α-Tocopherol from macrophages by the cAMP-inducible ABCA1 pathway.

We overexpressed ABCA1 in cells to determine whether this would enhance α-Tocopherol secretion. For these studies, we transfected BHK cells with an inducible ABCA1 gene, radiolabeled cells with [14C]-α-Tocopherol, induced ABCA1 expression, and measured apoA-I-mediated α-Tocopherol efflux. Control BHK cells contain virtually no detectable ABCA1, whereas induced cells express very high levels of this protein (Fig. 5A, inset). Based on immunoblots, this expression level is 5–10-fold higher than maximum levels in human fibroblasts or murine macrophages. ApoA-I promoted significant cholesterol and phospholipid efflux only from the induced cells (not shown). Inducing ABCA1 expression caused only a small increase in α-Tocopherol efflux in the presence of BSA alone. ApoA-I removed very little α-Tocopherol from control cells but markedly stimulated α-Tocopherol efflux from ABCA1 transfectants (Fig. 5A), indicating that apoA-I-mediated α-Tocopherol secretion requires expression of ABCA1.

Two possible mechanisms could account for the role of ABCA1 in transporting cellular α-Tocopherol to lipid-free apoA-I. First, ABCA1 could transfer phospholipids to apoA-I, generating acceptors for α-Tocopherol that diffuse from cells by ABCA1-independent processes. Second, ABCA1 could transfer α-Tocopherol directly along with other lipids to apolipoproteins. To distinguish between these mechanisms, we incubated unlabeled

![Fig. 2. Dose-dependent apoA-I-mediated efflux of α-Tocopherol from normal (NL) and Tangier disease (TD) fibroblasts.](http://www.jbc.org/)

![Fig. 3. Effects of different acceptors on α-Tocopherol efflux from normal and Tangier disease fibroblasts.](http://www.jbc.org/)

![Fig. 4. Comparison of α-Tocopherol and cholesterol (UC) efflux from control and cAMP-treated RAW264 macrophages.](http://www.jbc.org/)

![Fig. 5. Effect of forced expression of ABCA1 on α-Tocopherol efflux from BHK cells.](http://www.jbc.org/)
trol and ABCA1-transfected BHK cells for 4 h with apoA-I, transferred the media to [14C]α-TOH-labeled control cells (low ABCA1), and measured [14C]α-TOH efflux during the second incubations. Results showed that apoA-I had little ability to promote α-TOH efflux from control cells whether pre-incubated with cells expressing very low or high levels of ABCA1 (Fig. 5B). Therefore, under these incubation conditions, apoA-I did not acquire enough phospholipid mass by the ABCA1 pathway to become an acceptor for α-TOH that diffuses from cells. This makes it likely that ABCA1 participates directly in the transport of α-TOH from cells to apolipoproteins.

We also examined the possibility that ABCA1 may be involved in transporting α-TOH into cells. The results showed that there was no difference in the cellular uptake of [14C]α-TOH between normal and Tangier disease fibroblasts (data not shown). Moreover, none of the treatment protocols used in this study, which were inserted between the labeling and efflux incubations, had a significant effect on the cellular content of [14C]α-TOH. It appears therefore that ABCA1 mediates the unidirectional transport of α-TOH from cells to suitable extracellular acceptors.

DISCUSSION

Previous studies showed that lipid-free apoA-I promotes secretion of α-TOH from cells (11). Here we show that this occurs by the ABCA1 lipid secretory pathway. Three lines of evidence support this conclusion. First, treatment of human fibroblasts and murine RAW264 macrophages with cholesterol and/or 8-Br-cAMP, which induces ABCA1 expression and apoA-I-mediated lipid removal (16, 23), also enhanced apoA-I-mediated α-TOH efflux. Second, apoA-I lacked the ability to remove α-TOH from Tangier disease fibroblasts that have a nonfunctional ABCA1. Third, BHK cells that lack an active ABCA1 pathway in the basal state markedly increased secretion of α-TOH to apoA-I when forced to express ABCA1.

The current study shows that mature HDL particles are also capable of promoting α-TOH efflux from cells. Comparisons of normal and Tangier disease cells exposed to native and trypsin-treated HDL revealed that this efflux occurs by both ABCA1-dependent and -independent processes. The rate of diffusion of α-TOH from cells exceeds that observed for cholesterol, which may reflect the comparatively greater hydrophilicity of the vitamin. These findings indicate that lipoproteins can remove α-TOH from cells by both passive and active processes.

The diffusible properties of α-TOH raised the possibility that the effects of ABCA1 on α-TOH secretion were indirect. By lipidating apoA-I, ABCA1 may have generated acceptors for α-TOH that passively desorbs from the plasma membrane. Our pulse-chase studies, however, showed that exposing apoA-I to cells expressing high levels of ABCA1 did not increase its ability to remove α-TOH from cells lacking ABCA1. These findings indicate that, during the brief incubations used in these studies, ABCA1 did not lipidate apoA-I enough to generate acceptors for α-TOH that diffuses from cells. Thus, ABCA1 appears to be involved directly in transporting α-TOH. It is possible that ABCA1 co-transports α-TOH, cholesterol, and phospholipids to the cell surface where the lipid complex is solubilized and removed by apolipoproteins (12). ABCA1 plays a gatekeeper role in transporting tissue cholesterol into the HDL metabolic pathway (12). The current study suggests that this protein also functions to transport α-TOH taken up by tissues back into plasma via apoA-I and HDL. Although α-TOH distributes among all major lipoprotein subclasses, HDL is the preferred vehicle for transport of α-TOH to at least some tissues (3–6). This tissue uptake appears to utilize the SR-B1 receptor system (4), which also selectively transports cholesteryl esters from HDL into cells (7).

Expression of SR-B1 is suppressed when liver cells accumulate α-TOH, consistent with feedback repression of α-TOH delivery into cells by this pathway (29). We found that supplementing macrophages and fibroblasts with α-TOH had no effect on ABCA1 expression (data not shown), implying that α-TOH does not regulate its secretion by this transporter. Taken together, these studies suggest that SR-B1 and ABCA1 operate in concert to transport α-TOH between tissues by the HDL pathway.

ABCA1 is highly expressed in the liver, where it may play a role in secreting dietary α-TOH delivered to the liver from the intestine via apoB-containing lipoproteins. Hepatocytes contain α-TTP, which retains α-TOH, transports the vitamin between membranes, and promotes its secretion (9, 10). Because much of the α-TOH secreted from hepatocytes in vivo is associated with nascent VLDL, it was assumed that α-TTP facilitates incorporation of α-TOH into VLDL during its assembly and secretion. Arita et al. (10), however, showed that α-TTP stimulates secretion of α-TOH from cultured hepatocytes by a pathway distinct from lipoprotein assembly but linked to cholesterol metabolism and/or transport. Our results suggest that this pathway involves ABCA1 and suitable extracellular acceptors of α-TOH. The secreted α-TOH may then distribute among lipoproteins by a process facilitated by phospholipid transfer protein (2).

The ABCA1 pathway may also help generate α-TOH in tissues to alleviate oxidative stress. Macrophages ingest large amounts of cell-derived cholesterol and other lipids within tissues with high rates of cell turnover and at sites of inflammation. In the artery wall, macrophages take up oxidized lipoproteins, leading to atherogenic foam cell formation. Cholesterol accumulation in macrophages induces ABCA1 expression (30), which would be predicted to increase secretion of α-TOH along with cholesterol and phospholipids. In support of this idea is a study by Asmis and Jelk (31) showing that cholesterol loading of cultured macrophages depletes them of α-TOH. Whether this renders cells more susceptible to subsequent oxidation is not clear at present. Although some intracellular α-TOH may be localized to sites for release into the extracellular environment, other pools of α-TOH may protect cells against oxidation. The α-TOH transported from cells by ABCA1 may help protect co-secreted and other extracellular lipids from oxidation. Increased secretion of cellular α-TOH by lipid-laden macrophages could also help explain why lipoproteins in diseased artery walls contain relatively normal amounts of vitamin E despite the fact that their lipids are oxidized (32).

The current study shows that lipophilic compounds other than cholesterol and phospholipids utilize the ABCA1 pathway for secretion from cells. The robust induction of ABCA1 by sterols implies that these molecules are primary targets for secretion by this transporter. Our results suggest that secretion of at least some cellular pools of α-TOH is tightly linked to sterol metabolism and transport. It remains to be determined whether this also applies to other fat-soluble vitamins and lipophilic compounds.

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