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Heterogeneity of high density lipoprotein generated by ABCA1 and ABCA7

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Abstract The assembly of HDL by helical apolipoprotein and cellular lipid was studied using HEK293 cells to which ecdysone-inducible human ABCA1 or human ABCA7 was transfected. Expression of both ABCA1 and ABCA7 was induced linearly proportional to ponasterone A concentration in the medium. In the experimental conditions used, the ABC protein expression levels limited the rate of lipid release when the apolipoprotein concentration was high, and the apolipoprotein concentration was rate-limiting when the ABC protein expression levels were high. When ABCA1 expression increased in conditions in which it was rate-limiting, relative cholesterol content to phospholipid increased in the HDL produced. In contrast, it was constant when ABCA7 expression increased. To investigate the background mechanism, the HDL particles were analyzed by density gradient ultracentrifugation and high performance lipid chromatography. The ABCA1-mediated reaction produced two distinct HDLs, large cholesterol-rich and small cholesterol-poor particles, and the ABCA7-mediated reaction generated mostly small cholesterol-poor particles. The increase of HDL assembly with the increase of ABCA1 expression was predominant in large cholesterol-rich particles, whereas only small cholesterol-poor HDL increased as ABCA7 expression increased. We conclude that ABCA1 generates cholesterol-rich and cholesterol-poor HDL and that the former is more prominently dependent on the increase of ABCA1 expression. ABCA7 produces this HDL subfraction only as a very minor component.—Hayashi, M., S. Abe-Dohmae, M. Okazaki, K. Ueda, and S. Yokoyama. **Heterogeneity of high density lipoprotein generated by ABCA1 and ABCA7.** ***J. Lipid Res.*** **2005.** 46: **1703–1711.**

Supplementary key words ATP binding cassette transporter A1 • ATP binding cassette transporter A7 • cholesterol • ecdysone-inducible

ABCA1 mediates the assembly of HDL with extracellular helical apolipoprotein and cellular lipid (1). This reaction is the major source of plasma HDL (2–4) and one of the rate-liming reactions for the regulation of its level (5, 6). The reaction mediates the generation of HDL particles with apolipoprotein, primarily recruiting cellular phospholipid (7). Cholesterol content in these particles is independently regulated by various cellular factors, potentially including protein kinase C and related signaling machineries (8–10), caveolin-1 (11), acyl-CoA:cholesterol acyltransferase (10, 12), and also perhaps other factors relating to intracellular cholesterol trafficking pathways (13). When ABCA1 is transfected to HEK293 cells, which otherwise do not express ABCA1, phospholipid and cholesterol are both released and cholesterol-rich HDL is generated upon incubation of the cells with apolipoprotein A-I (apoA-I) (14–16). ABCA7 also mediates the generation of HDL with apolipoproteins when transfected to HEK293 cells, but the relative cholesterol content to phospholipid in the HDL was lower than that produced by the ABCA1-mediated reaction (16, 17). The relative increase of cholesterol release seemed greater than that of phospholipid when ABCA1 protein level was upregulated by dibutyryl cAMP and phorbol ester (16), so we wondered whether the expression level of ABCA proteins is also a factor that regulates cholesterol content in the HDL. There are many reports that ABCA1 expression can be induced by various factors such as cAMP analogs (18–20), phorbol ester (10, 11), and ligands of liver X receptor or retinoid X receptor, including alteration of cellular cholesterol level (21–24). However, these compounds influence other cellular conditions and sterol metabolism and may not be suitable for examining the isolated effect of the expression level of the ABCA proteins. To address this question,

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Abbreviations: apoA-I, apolipoprotein A-I; DF, 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium; GFP, green fluorescent protein.

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we used the ecdysone-inducible mammalian expression system (Invitrogen), based on the ability of the insect molting hormone 20-H ecdysone to activate gene expression via the ecdysone receptor (25). Mammalian cells are not responsive to ecdysone or its analog ponasterone A, so the effect of these reagents is minimized other than the expression of a specific gene designed to react. With these cell systems, the expression of ABCA1 and ABCA7 was regulated quantitatively and the generation of HDL was examined accordingly.

Interestingly, the cholesterol content in HDL increased in a dose-dependent manner with ABCA1 but not with ABCA7. The ABCA1-mediated reaction produced large cholesterol-rich and small cholesterol-poor HDL particles, and the former predominantly increased as ABCA1 increased. On the other hand, ABCA7 produced predominantly small cholesterol-poor HDL even when its expression increased.

MATERIALS AND METHODS

Apolipoproteins

ApoA-I and apoA-II were isolated from fresh human HDL (26) and dissolved (19) in phosphate-buffered saline as stock solutions (1 mg/ml) to be stored at 4°C as described elsewhere.

DNA construction and transfection

Full-length cDNAs for human ABCA1 and human ABCA7 were cloned as described previously (15, 16) and subcloned into pIND vector (Invitrogen). ABCA1 cDNA within pEGFP-N1 was digested with *NotI* and ABCA7 cDNA within pEGFP-N1 was digested by *NotI* and *Hind*III. The purified inserts were ligated into pIND to obtain pIND-ABCA1-green fluorescent protein (GFP) and pIND-ABCA7-GFP. HEK293 expressing ecdysone receptor was purchased from Invitrogen and maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (DF) supplemented with 10% (v/v) FBS (Hyclone) containing 400 μg/ml Zeocin (Invitrogen). The cloned cDNAs were transfected with Lipofectamine PLUS reagent (Invitrogen) according to the manufacturer's recommendation. Cells permanently introduced were selected with G418, and clones with higher level expression of the fusion proteins were further selected by FACStar (Becton Dickinson).

Evaluation of ABCA1 and ABCA7 expression levels

Total membrane fraction was prepared, and the expression of ABCA1 and ABCA7 was analyzed by immunoblotting using rabbit polyclonal anti-GFP antibody (Molecular Probes) (16).

Cellular lipid-release assay

Cells were seeded onto six-well trays at a density of 1.4×10^6 cells/well with 10% FBS-DF medium and incubated for 24 h. After treatment with ponasterone A of the ABCA1- and ABCA7-transfected cells for 17 and 24 h, respectively, cellular lipid release was induced in the presence of apoA-I or apoA-II (1–20 μg/ml) in DF containing 0.02% BSA and ponasterone A. Lipid was extracted from the medium, and free cholesterol and choline-phospholipid were measured in separate enzymatic assay systems (19).

Density gradient analysis

Cells were subcultured on 100 mm dishes at a density of 8.0×10^6 cells/dish, treated with ponasterone A, and stimulated by apoA-I (2 or 10 μg/ml) for 24 h in 5 ml/dish DF medium containing 0.02% BSA. The media from two dishes were combined and centrifuged to remove cell debris, and 8 ml of the supernatant was processed for sucrose density gradient ultracentrifugation (27). The solution was collected from the bottom of the tube

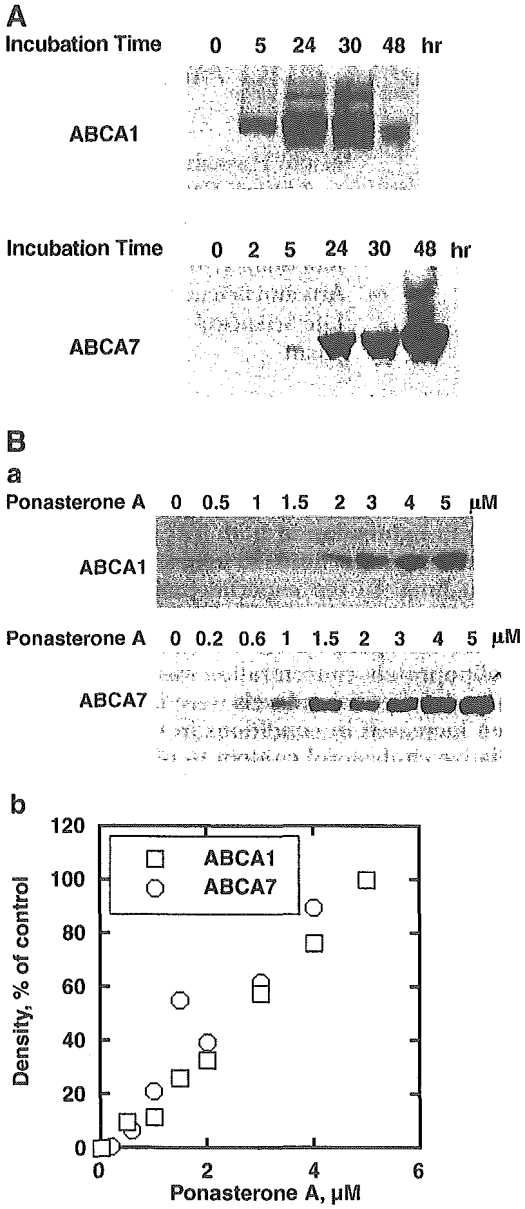

Fig. 1. Induction of green fluorescent protein (GFP)-tagged ABCA1 or ABCA7 expression by ponasterone A. A: The ecdysone-inducible cells were cultured for 24 h and incubated for the indicated times in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium containing 10% FBS in the presence of 5 μM ponasterone A. The membrane fraction (100 μg of protein) was analyzed for ABCA1 or ABCA7 by Western blotting against GFP. B: The ecdysone-inducible cells were cultured for 24 h and incubated for 17 h (ABCA1) or 24 h (ABCA7) in the presence of various concentrations of ponasterone A. The membrane fraction (100 μg of protein) was analyzed for ABCA1 or ABCA7 by Western blotting. The graph represents the density of the bands from the immunoblot quantitated by digital scanning in an Epson GT9500. The results are expressed as percentage of control (5 μM ponasterone A) for ABCA1 and ABCA7 (squares and circles, respectively).

into 13 fractions. The cholesterol and choline-phospholipid contents as well as the density were determined for each fraction (19).

Lipoprotein analysis by HPLC

Lipoproteins in the conditioned medium were analyzed with an HPLC system (28) at Skylight Biotech, Inc. (Akita, Japan). The conditioned medium was centrifuged at 10,000 rpm for 5 min to remove cell debris, and a 200 μl aliquot was applied for an HPLC system using two tandem gel permeation columns (Lipopropak XL; 7.8 mm × 300 mm; Tosoh) with 0.05 M Tris-buffered acetate, pH 8.0, containing 0.3 M sodium acetate, 0.05% sodium azide, and 0.005% Brij-35 at a flow rate of 0.7 ml/min, and an online enzymatic lipid detection system. The method was thoroughly validated against the reference methods of ultracentrifugation and of Superose gel permeation chromatography, including the criteria of subfraction analysis of HDL (29).

RESULTS

Induction of ABCA1 and ABCA7 by ponasterone A

GFP-tagged ABCA1 or ABCA7 cDNA with the ecdysone-inducible mammalian expression system was transfected to HEK293 cells that stably express the ecdysone receptor. It was previously confirmed that attachment of GFP to the C terminus of ABCA1 or ABCA7 did not influence their ability to produce HDL (16, 30). Expression of the ABCA proteins was examined by immunoblotting against GFP. With 5 μM ponasterone A treatment, expression of the GFP-tagged protein became apparent within 5 h, and it increased for 24 h in ABCA1 cells and for 48 h in ABCA7 cells (**Fig. 1A**). Therefore, 24 h induction was chosen as the optimum condition for ponasterone A to induce ABCA1 and ABCA7 for comparison. The dose-dependence of ABCA1 or ABCA7 expression was examined using various concentrations of ponasterone A. The immunoreacting bands of ABCA1-GFP and ABCA7-GFP both linearly increased ponasterone A in a dose-dependent manner (R^2 = 0.99 and 0.94, respectively) (Fig. 1B). At higher concentrations of ponasterone A (>10 μM), the expression levels seemed to reach a maximum (data not shown). The apoA-I-mediated lipid release increased by ponasterone A in a dose-dependent manner (**Fig. 2**). ABCA7 mediated cholesterol release less than ABCA1 with respect to its relative amount of phospholipid release.

Characterization of the lipid release mediated by ABCA proteins and apolipoproteins

Figure 3 demonstrates the release of cholesterol and phospholipid from HEK293 cells by apoA-I in the presence of ABCA1 and ABCA7. Cholesterol was almost all in a free form, and the amount of cholesteryl ester was negligible. When ABC protein expression is high (at high ponasterone A concentrations), apoA-I concentration limits the rate of lipid release. On the other hand, the expression levels of ABCA proteins limit the rate with the ponasterone A concentration used when apoA-I concentration is 10 and 20 μg/ml. **Figure 4** shows the results of similar experiments with apoA-II. The release of cholesterol and phospholipid by apoA-II appeared similar to the results

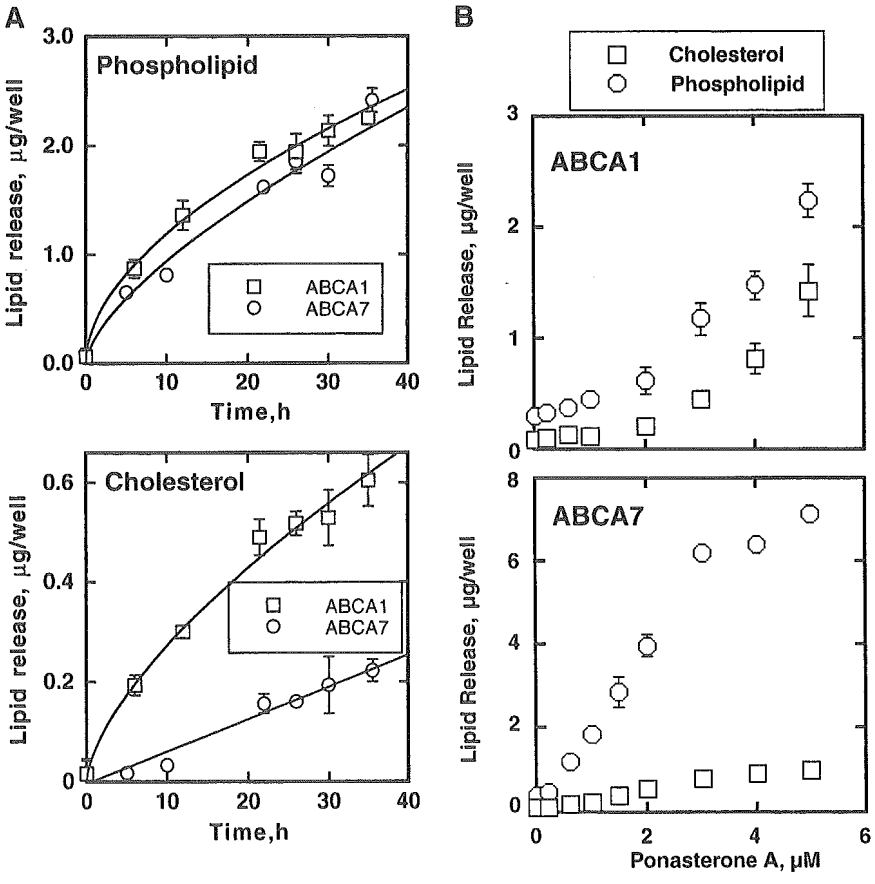

Fig. 2. Apolipoprotein A-I (apoA-I)-mediated release of cholesterol and choline-phospholipid. The ecdysone-inducible cells were incubated with the indicated concentrations of ponasterone A for 17 or 24 h. After washing with buffer H, the cells were incubated with apoA-I. A: Time-dependent profiles of the lipid release at 5 μM ponasterone A. B: Lipid release by apoA-I for 24 h at various concentrations of ponasterone A. Cholesterol (squares) and choline-phospholipid (circles) were determined in the medium. Data represent means ± SD for three samples.

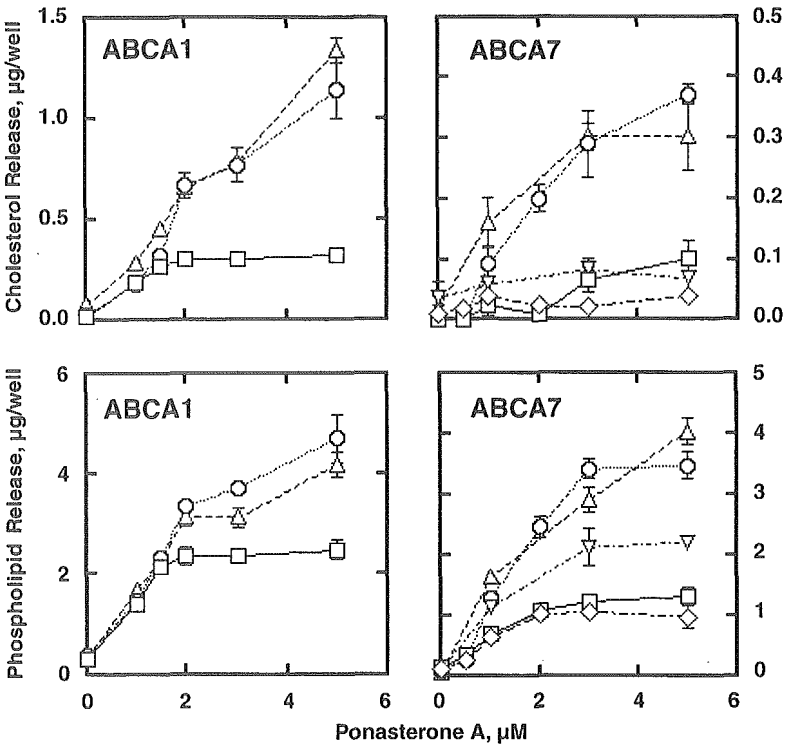

Fig. 3. Lipid release by apoA-I mediated with ABCA1 or ABCA7. The cells were induced for the expression of ABCA1 or ABCA7 in the presence of ponasterone A at 17 and 24 h, respectively. After washing, the cells were incubated with various concentrations of apoA-I [1, 2, 5, 10, and 20 μg/ml (diamonds, squares, inverted triangles, circles, and triangles, respectively)] for 24 h. Cholesterol and choline-phospholipid were measured in the medium. Total cellular cholesterol and choline-phospholipids were 17.5 ± 0.7 and 91.2 ± 2.5 μg/well in the ABCA1-expressing cells and 24.1 ± 2.5 and 116.2 ± 5.8 mg/well in the ABCA7-expressing cells (average ± SD for six samples).

with apoA-I with respect to rate-limiting profiles. When apoA-II concentration is as low as 1 and 2 μg/ml, it limits the rate of lipid release. On the other hand, ABCA1 or ABCA7 limits the rate when apoA-II concentration is as high as 5 and 10 μg/ml.

In the conditions in which ABCA proteins are rate-limiting, the ratio of cholesterol to phospholipid was examined in the released lipid (**Fig. 5**). When the expression of ABCA1 increases by increasing ponasterone A from 1 to 5 μM, the relative content of cholesterol in the released lipid increased by both apoA-I and apoA-II, from 0.1 to 0.4 and from 0.04 to 0.17, respectively (Fig. 5). In contrast, it remained constant at a low level when the expression of ABCA7 increased (Fig. 5).

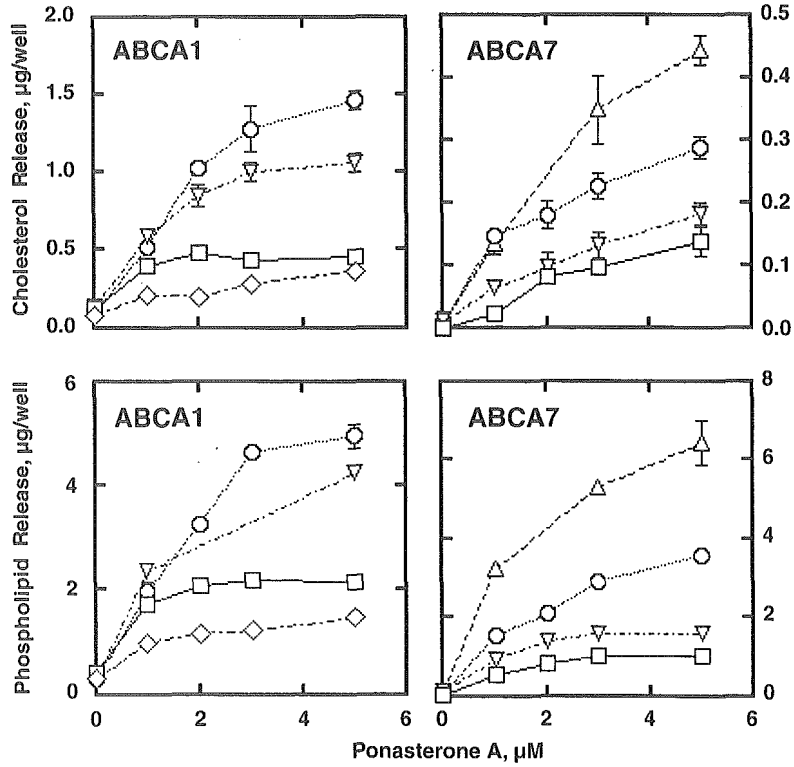

Fig. 4. Lipid release by apoA-II mediated with ABCA1 or ABCA7. The cells were induced for ABCA1 or ABCA7 in the presence of ponasterone A at 17 and 24 h, respectively. After washing, the cells were incubated with apoA-II [1, 2, 5, 10, and 20 μg/ml (diamonds, squares, inverted triangles, circles, and triangles, respectively)] for 24 h. Cholesterol and choline-phospholipid were determined in the medium.

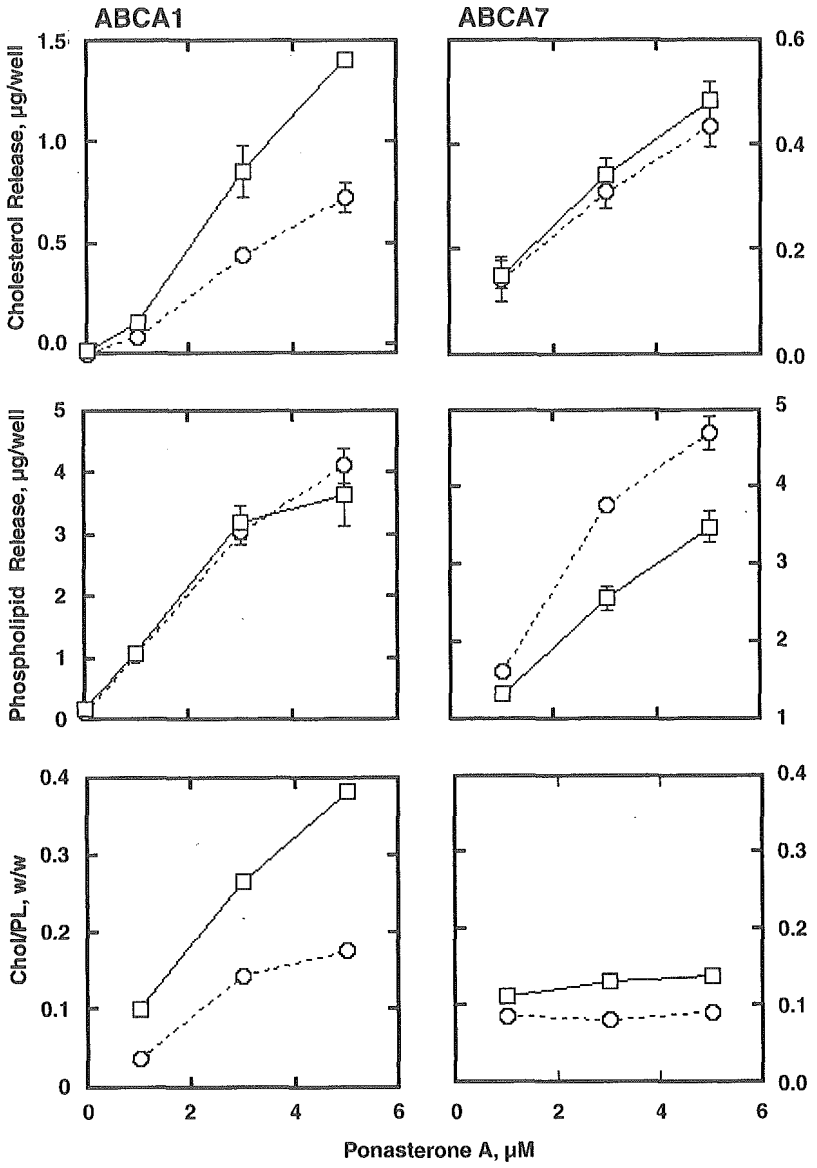

Fig. 5. Cholesterol-phospholipid ratio in the lipid released by apoA-I and apoA-II (squares and circles, respectively) mediated with ABCA1 or ABCA7. Expression of ABCA1 or ABCA7 was induced by ponasterone A, and cellular lipid release was induced by apolipoprotein (10 μg/ml). The ratio of cholesterol to phospholipid (Chol/PL) in mass is shown in the two bottom panels.

Density gradient ultracentrifugation analysis of the conditioned media

The conditioned media with apoA-I of HEK293 cells transfected with ABCA1 or ABCA7 were analyzed by density gradient ultracentrifugation. Both cholesterol and phospholipid were recovered in the fractions, with a density range of HDL to confirm that the lipid was released to form HDL particles. The density of the peak was 1.08 g/ml with the ABCA1-transfected cells and 1.10–1.11 g/ml with the ABCA7-transfected cells (**Fig. 6**), consistent with our previous observation (16). Density profiles of lipid distribution for the ABCA1-transfected cells appear with a shoulder at ~1.11 g/ml, whereas those for the ABCA7-transfected cells were rather symmetric, with a slight irregularity in the lower density side.

Analysis of the media by HPLC

To examine the heterogeneity of the HDL generated, the conditioned media were analyzed by HPLC with a gel permeation column. Elution profiles of cholesterol and phospholipid were monitored online. **Figures 7** and 8 show the results with the apoA-I-conditioned media of the ABCA1- and ABCA7-transfected cells. The medium of the ABCA1-transfeted cells contained two peaks: large cholesterol-rich particles with a diameter of 13 nm, and small cholesterol-poor particles with a diameter of 10 nm. In contrast, the ABCA7-transfected cells generated a predominant peak that is small and cholesterol-poor, accompanied by large particles as a very minor component. These profiles were essentially the same in the conditioned media of HEK293 cells to which ABCA1 or ABCA7 was transiently expressed without the GFP tag (data not shown). When the lipid release was increased either by increasing ABCA1 expression (Fig. 7) or by increasing apoA-I (Fig. 8), a more prominent increase was induced in the large cholesterol-rich particles, and accordingly, the relative amount of cholesterol to phospholipid in the media increased.

In contrast, small cholesterol-poor particles were still major components of the HDL generated by the apoA-I-ABCA7-mediated reaction. This profile did not change even when ABCA7 expression was increased, resulting in

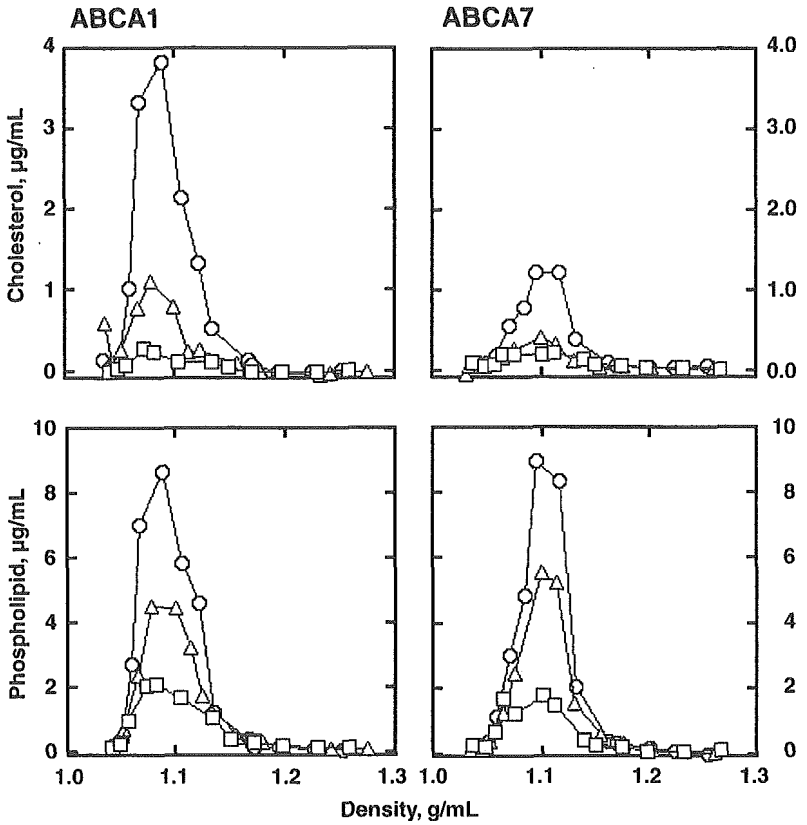

Fig. 6. Density gradient analysis of the lipids released by ABCA1- or ABCA7-expressing cells. The cells were induced for the expression of ABCA1 or ABCA7 by incubating with 5 mM ponasterone A for 17 and 24 h, respectively, and cellular lipid was released by apoA-I [2, 4, and 10 μg/ml (squares, triangles, and circles, respectively)] for 24 h. The medium was analyzed by density gradient ultracentrifugation as described in Materials and Methods. Each fraction was determined for density and concentration of cholesterol and phospholipid.

the relative cholesterol content remaining constantly low (Fig. 7). The same tendency was observed when apoA-I was rate-limiting and increased the generation of HDL from the ABCA7-transfeted cells (Fig. 8).

Component analysis of the peaks (31) estimated that the large HDL particles generated by the ABCA1-apoA-I reaction contained cholesterol with 35–38% weight of phospholipid and that the small HDL particles contained

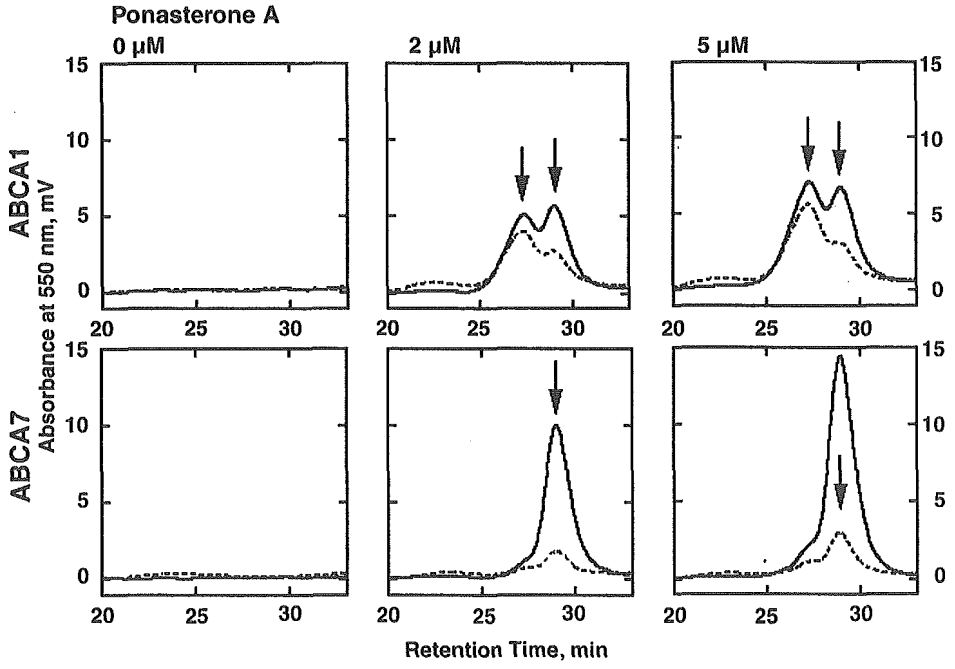

Fig. 7. HPLC analysis of the lipid released by ABCA1- or ABCA7-expressing cells. Lipoprotein analysis of the culture medium of ABCA1- or ABCA7-induced HEK293 cells by HPLC. The cells were induced for the expression of ABCA1 or ABCA7 by incubating with ponasterone A (2 and 5 μM) for 17 and 24 h, respectively, and cellular lipid was released by apoA-I (10 μg/ml) for 24 h. The conditioned medium (200 μl) was analyzed by the HPLC lipoprotein analysis system by monitoring cholesterol (broken lines) and phospholipid (solid lines). The arrows indicate eluting positions of the particles with diameters of 13 and 10 nm.

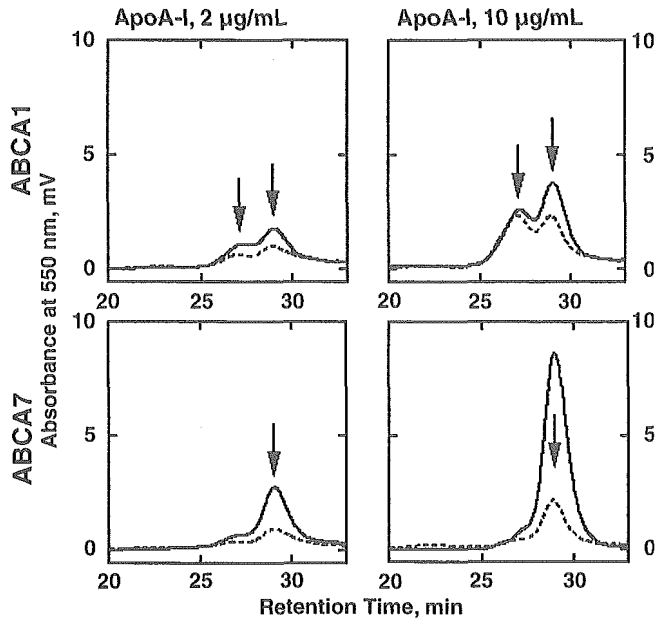

Fig. 8. HPLC analysis of the lipid released by ABCA1- or ABCA7-expressing cells. The cells were induced for the expression of ABCA1 or ABCA7 by incubating with ponasterone A (5 μM) for 17 and 24 h, respectively, and cellular lipid was released by apoA-I (2 and 10 μg/ml) for 24 h. The conditioned medium (100 μl) was analyzed by the HPLC lipoprotein analysis system by monitoring cholesterol (broken lines) and phospholipid (solid lines). The arrows indicate eluting positions of the particles with diameters of 13 and 10 nm.

10–13%. A major HDL component generated by the ABCA7-apoA-I reaction contained 8–11%.

DISCUSSION

ABCA1 is a key cellular factor for the generation of plasma HDL (2–6). ABCA7 was also demonstrated to mediate a similar reaction to ABCA1 to generate HDL in vitro (16, 17). There are several differences between the ABCA1- and ABCA7-mediated reactions with respect to cholesterol content in the HDL generated and posttranscriptional regulation. Recent studies indicated that ABCA7 does not play a major role in lipid release from macrophages (32, 33). However, it may contribute to a source of plasma HDL, at least in female mice (33), and it may be involved in lipid metabolism in kidneys (32) and adipose tissues (33).

The levels of expression of ABCA proteins may also affect these reactions, which could have significant influence on the nature of the products, such as their cholesterol content. However, the regulation of expression of ABCA proteins is multifactorial, including the liver X receptor/retinoid X receptor system (21–24), cAMP (18–20), the calcium-signaling pathway (34), and the peroxisome proliferator-activated receptor α-related system (35, 36). The content of cholesterol in the product HDL is regulated somewhat independently of the HDL assembly reaction itself, potentially with the involvement of such factors as caveolin-1 (11), protein kinase C-related signals (8–10), and intracellular cholesterol level and its esterification (10, 12). To characterize the HDL assembly reaction by ABCA proteins, it is thus important to control the expression level of these proteins without influencing other cellular factors.

We established the HEK293 cell systems with ecdysone-inducible ABCA1 and ABCA7. Both proteins are tagged with GFP, which was shown previously not to interfere with the function of either protein and enabled us to estimate the protein expression levels on a common immunoreactivity basis.

The results of the experiments are summarized below. Expression of ABCA1 and ABCA7 were both linearly proportional to the concentration of ponasterone A in the culture medium. The release of cellular cholesterol and phospholipid is limited by both ABCA protein expression levels and extracellular apolipoprotein levels. When ABCA proteins are rate-limiting, cholesterol release relative to phospholipid increased as ABCA1 expression increased, whereas it remained constant when ABCA7 expression increased. Increase of ABCA1 expression and apolipoprotein concentration both resulted in the increase of cholesterol content in HDL, and it was attributed to expansion of production of this component. On the other hand, ABCA7 mediated the generation of HDL particles that are predominantly small and cholesterol-poor. Thus, ABCA1 is more effective in releasing cellular cholesterol than is ABCA7 by producing large cholesterol-rich HDL. Both ABCA1 expression and apolipoprotein concentration increased relative to the release of cholesterol to phospholipid, and this was attributable to the expansion of production of this component.

Heterogeneity in nascent HDL particles produced by cell-apolipoprotein interaction has been reported in a previous study. When CHO-C19 cells were incubated with 8 μg/ml apoA-I for 24 h, 9.0 and 11.2 nm particles were produced (37). In another experiment with J774 macrophages, incubation with apoA-I led to the formation of more than one type of apoA-I-containing lipid particles, 9 and 12 nm in diameter, having cholesterol-phospholipid ratios of 1:1 to 1:3 (mol/mol), respectively (38). We also reported density profiles of HDL generated by peritoneal macrophages and apoA-I, which suggested similar heterogeneity of the HDL particles (8). The physicochemical and molecular bases for the size heterogeneity of discoidal HDL can be discussed (39), but it is not clear how such a mechanism can be applied when HDL is organized upon the apolipoprotein interaction with ABCA1 and membrane lipid. Lipid composition and particle size are related to the structure of specific domains of apoA-I on HDL (40). ABCA1 may induce specific modification of apoA-I conformation, either directly or indirectly, and such a change may cause parallel changes in its ability to bind to phospholipid bilayers and to the integration of cholesterol (41).

A higher expression level of ABCA1 causes the increase of large cholesterol-rich HDL particles. Although the exact mechanism by which ABCA1 functions in the membrane is not known, a few interesting findings have been

reported. ABCA1 is said to alter the microenvironment of the plasma membrane and influence lipid-protein complex formation in the membrane (42). It forms an oligomeric structure and is related to its function for the biogenesis of nascent apoA-I-containing HDL (43). If oligomeric ABCA1 produces cholesterol-rich HDL, it is consistent with the view that a higher expression of ABCA1 and therefore an increase of oligomeric ABCA1 in the membrane may cause more production of cholesterol-rich particles. The apparent biphasic increase of the lipid release by the increase of ABCA1 expression (Fig. 2B) may also be consistent with this view. ABCA7 may not act in such a manner, and production of cholesterol-poor HDL simply increased when its expression increased. Tall and colleagues (32) suggested that the small amount of cholesterol release by the ABCA7-mediated reaction is attributable to its nonspecific acquisition by the phospholipid-HDL. It is not clear whether the small HDLs demonstrated in the present study gain cholesterol only by such a mechanism.

We have demonstrated a fundamental difference between ABCA1-mediated and ABCA7-mediated HDL assembly. ABCA1 mediates two types of HDL particles, large cholesterol-rich and small cholesterol-poor particles, and the generation of large cholesterol-rich particles is perhaps responsible for the efficient release of cell cholesterol. ABCA7 mediates the generation of only the latter type of particle. At higher expression, ABCA1 produces predominantly more large cholesterol-rich particles, whereas ABCA7 increases the production of only small cholesterol-poor HDL. The results presented here provide a new biochemical basis for understanding an HDL assembly pathway that involves ABCA1 or ABCA7.

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Assembly of High-Density Lipoprotein

Shinji Yokoyama

Abstract—Mammalian somatic cells do not catabolize cholesterol and need to export it for its homeostasis at the levels of cells and whole bodies. This reaction may reduce intracellularly accumulated cholesterol in excess and would contribute to prevention or regression of the initial stage of atherosclerosis. High-density lipoprotein (HDL) is thought to play a main role in this reaction, and 2 independent mechanisms are proposed for this reaction. First, cholesterol is exchanged in a nonspecific physicochemical manner between cell surface and extracellular lipoproteins, and cholesterol esterification on HDL provides a driving force for net removal of cell cholesterol. Second, apolipoproteins directly interact with cells and generate HDL by removing cellular phospholipid and cholesterol. This reaction is a major source of plasma HDL and is mediated by a membrane protein, ABCA1. Lipid-free or lipid-poor helical apolipoproteins primarily recruit cellular phospholipid to assemble HDL particles, and cholesterol enrichment in these particles is regulated independently. ABCA1 is a rate-limiting factor of the HDL assembly and is regulated by transcriptional factors and posttranscriptional factors. Posttranscriptional regulation of ABCA1 includes modulation of its calpain-mediated degradation. (*Arterioscler Thromb Vasc Biol.* 2006;26:20-27.)

Key Words: HDL ■ ABCA1 ■ apoA-I ■ apoE ■ cholesterol

Cholesterol is an essential constituent of the cell membrane and regulates its functions. It controls general fluidity of the membrane lipids, and more importantly, forms cluster domains with sphingolipids to accommodate specific membrane proteins, such as those related to signal transduction. Cholesterol is also important as a precursor of steroid hormones and bile acids but cannot be converted to energy. Biosynthesis of cholesterol is most active in the liver in vertebrates, but it is carried out in all of the somatic cells, taking a complicated process involving ≥20 enzymes that requires 3 ATP and 7 reduced nicotinamide-adenine dinucleotide phosphate molecules. In contrast, the catabolism of cholesterol is very limited in peripheral cells. Most of the cholesterol molecules in the body of vertebrates are transported to the liver and converted to bile acids that are extensively reused in the enterohepatic circulation. This scheme of cholesterol metabolism indicates that our system recognizes it as an important and valuable molecule that should not be wasted at all. Accordingly, we are well prepared for crisis management of a cholesterol shortage but very poorly prepared for its overload.

The release of cholesterol from somatic cells is, thus, an important part of cholesterol homeostasis. In the past several years, significant progress has been made in understanding the mechanism of cellular cholesterol release.

Cholesterol transport from somatic cells to the liver is mediated by HDL. This pathway is under kinetic control and in a steady state by assembly and clearance of plasma lipoproteins and extracellular cholesterol metabolism mediated by lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein, and other active molecules.[1] This pathway is often referred to as a concept of "reverse cholesterol transport" in relation to an antiatherosclerosis potential of HDL, which is based on the 2 lines of evidence that the plasma HDL level is negatively correlated with the risk increase of atherosclerotic vascular diseases,[2] and HDL reduces the cellular cholesterol in vitro.[3]

The most critical step of "reverse cholesterol transport" seems to be the release of cholesterol from cells, and 2 major mechanisms are proposed for this step.[4–7] One is nonspecific cholesterol efflux from the cellular surface by physicochemical cholesterol exchange between cell membrane and extracellular "acceptors." The net release of cellular cholesterol is driven by extracellular cholesterol esterification by LCAT. HDL plays a major role in this pathway by presenting its large capacity for cholesterol accommodation and by providing a major and optimum site for the LCAT reaction. Cellular factors may also modulate this reaction. Scavenger receptor B1 seems to enhance the cholesterol exchange rate between the cell membrane and HDL perhaps through a specific mode of binding to HDL.[8,9] ATP-binding cassette transporter (ABC) G1/4 alters intracellular cholesterol distribution to increase its release by this pathway.[10]

The other mechanism for cellular cholesterol release is the generation of new HDL particles with cellular phospholipid and cholesterol. This reaction is mediated by the interaction of helical apolipoproteins with cells. ABCA1 is a key cellular

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factor for this reaction, and it is a major source of plasma HDL.

HDL assembly by cellular lipid and extracellular helical apolipoproteins was first described as the activity of apolipoproteins of HDL removing phospholipid and cholesterol from mouse peritoneal macrophages and generating new HDL particles.[11] The lipoprotein thus generated met the criteria of prebeta-HDL with respect to physical and chemical properties,[11] morphological appearance,[12,13] and reactivity to LCAT[14,15]. Various helical apolipoproteins with amphiphilic helical segments can carry out the reaction, as can synthetic amphiphilic peptides.[16]

Although the rate of cholesterol release is much higher than phospholipid in the nonspecific lipid exchange reaction, apolipoprotein primarily recruits phospholipid rather than cholesterol in its reaction to form HDL particles.[17] HDL generated by this reaction largely contains phospholipid and unesterified cholesterol, and LCAT reaction helps maturation of this HDL as it generates core cholesteryl ester.[14,15] However, unlike cholesterol release by nonspecific exchange reaction, cholesterol esterification does not induce additional cellular cholesterol release when the HDL generated is already cholesterol rich.[14]

Tangier Disease and ABCA1: Apolipoprotein-Mediated HDL Assembly Is a Major Source of Plasma HDL

Tangier disease is a genetic disorder known as one of the familial HDL deficiencies.[18] The cells from the patients with this disease were shown to be impaired in the interaction with apolipoprotein and lacking the HDL assembly.[19,20] Mutations were identified in the gene of ABCA1 in this disease and other types of familial HDL deficiencies,[21–26] and disruption of this gene resulted in HDL deficiency in mice. Thus, ABCA1 was shown to be essential for the production of plasma HDL.[27,28] Although the Tangier cells do not interact with apolipoproteins and do not generate HDL,[19,20] they are intact with respect to nonspecific exchange–based cholesterol release.[19] This means that ABCA1-mediated reaction is specific and biological.

When cAMP induces expression of ABCA1 in RAW264 cells, it also induces apolipoprotein A-I (apoA-I) binding and HDL assembly.[29,30] Chemical cross-linking studies demonstrated a direct interaction of helical apolipoproteins with ABCA1 that was transfected to the cells.[31,32] Thus, ABCA1 functions as a mediator for apolipoprotein cell binding, and direct interaction between the 2 molecules is implicated. Hot spots for ABCA1 mutation to cause its dysfunction are in the cytosolic ATP–binding cassette domains and the cell surface domains, according to the model proposed,[33] so that the direct interaction of ABCA1 with extracellular apolipoproteins may be an essential part of its function.

The ABCA1-mediated HDL biogenesis is a major source of plasma HDL. It is, however, of interest that ABCA7 also mediates the HDL assembly in vitro in a very similar manner to ABCA1.[34,35] When ABCA1 or ABCA7 is transfected to HEK 293 cells, the former produced large cholesterol–rich and small cholesterol–poor HDL, but the latter generated only small cholesterol–poor HDL on the interaction with apoA-I,[36] showing a fundamental difference between the HDL assembly mediated by these proteins. This reaction may be important locally as a backup system for ABCA1 but may not significantly contribute to the regulation of plasma HDL concentration.[37]

The lipid-lowering drug probucol has been known for its strong antilipid-oxidative nature, which thereby provided a background for the interpretation of its clinical effect of reducing cutaneous xanthomas[38] and the prevention of atherosclerosis in animal experiments.[39,40] However, probucol also decreases HDL,[41] and this apparent conflict made this drug very controversial.[42] Interestingly, probucol inhibits apolipoprotein–cell interaction and generation of HDL with cellular lipids both in vitro[43,44] and in vivo.[45] This is very similar to the finding with the cells of Tangier disease. The kinetics of HDL metabolism in the probucol-fed mice are also similar to those observed with Tangier patients.[45] The findings with probucol also supported the view that the ABCA1-mediated biogenesis of HDL is the main source of plasma HDL.

Assembly of HDL Particles and Their Cholesterol Enrichment

HDL-like particles are generated in vitro with helical apolipoproteins and phospholipid, with or without core lipid and cholesterol.[46] The reaction does not require specific catalysts except for energy to disperse these molecules to homogeneity, and the lipoprotein particles thus generated contain at least a few hundred phospholipid molecules. This means that HDL-like particles are thermodynamically stable molecular assemblies of helical apolipoproteins and phospholipid. In contrast, the cholesterol-apolioprotein complex can never be generated in such a manner. If a similar physicochemical nature should be applied for the ABCA1/apolipoprotein -mediated assembly of HDL, "lipidation" of apolipoprotein is likely to take place primarily with phoshpolipid, and it should be in a snap-in manner rather than "gradual growth." In fact, HDL particles are generated primarily with membrane phoshpolipid by this reaction as discussed below.

Many cells interact with apolipoprotein and produce cholesterol-rich HDL, such as macrophages and most fibroblasts,[11,12,14,29,47–49] but some cells produce only cholesterol-poor HDL.[48,50] The third group of cells do not interact with apolipoprotein and produce no HDL, represented by Tangier cells.[19,20,48] Expression of ABCA1 is a necessary factor for the generation of HDL whether cholesterol-rich or -poor,[48] so that ABCA1 primarily mediates the interaction of apolipoprotein and membrane phospholipid to generate HDL particles. Enrichment of cholesterol in the generated HDL may be an independent process.[48,50–52]

Cholesterol enrichment in HDL can be regulated by the modulation of cellular factors. Treatment of the rat vascular smooth muscle cells with cytokines and PKC (protein kinase C) activators increased cholesterol in HDL generated by apolipoprotein.[50] In contrast, treatment of macrophages and the above-conditioned vascular smooth muscle cells with PKC inhibitors decreased the cholesterol content in HDL.[50,51] Cellular cholesterol levels may also contribute to the regulation of cholesterol content in the HDL produced.[53,54]

Intracellular Cholesterol Mobilization for HDL Assembly and Triggering Signals

Stimulation and inhibition of PKC modulates cholesterol content in the HDL generated by apolipoprotein–cell interaction as mentioned above, and the intracellular cholesterol compartment used for esterification by acylCoA: cholesterol acyltransferase (ACAT) decreased reciprocally to the increase of HDL cholesterol content.[50,51] Cholesterol release by the apolipoprotein is almost linear up to 24 hours, whereas the decrease of the ACAT-available cholesterol compartment rapidly reaches maximum in a few hours.[51] In contrast, no such rapid and dramatic change takes place when cell cholesterol is removed by nonspecific exchange.[50] Therefore, a specific intracellular signal rather than a mere general decrease of membrane cholesterol seems to trigger mobilization of cholesterol. Other reports may be consistent with this hypothesis, such that HDL activates PKC,[55] Fab fragments of anti–apoA-I antibody inhibit the removal of cholesterol from the intracellular pool but not from plasma membrane,[56] and fibroblasts of Tangier disease patients showed impairment of intracellular cholesterol removal by HDL.[19,57] It is interesting to note that the most extreme case of the decrease of this compartment was by stimulation of the cells with peroxidase-treated HDL[58,59] in which apoA-I/A-II heterodimer, presented as a complex with lipids, is responsible for this stimulation.[60]

Rat astrocytes secrete cholesterol-rich HDL by endogenous apolipoprotein E (apoE) and cholesterol-poor HDL by exogenous apolipoproteins including apoA-I and apoE.[61] Interaction of cholesterol with sphingomyelin in plasma membrane was shown to be a factor in the regulation of cholesterol content in the apoA-I–generated HDL, so that a sphingomyelin/cholesterol-rich membrane domain is a candidate compartment selectively used by the HDL assembly.[62,63] The involvement of caveolin-1, a molecule related to intracellular cholesterol trafficking to a specific membrane domain, such as caveola rich in cholesterol and sphingomyelin, was demonstrated[64–67] in the cholesterol export to HDL[66] and, more specifically, in cholesterol trafficking to the apolipoprotein-mediated HDL generation.[49] ApoA-I induces rapid translocation of caveolin-1 and cholesterol to cytosolic lipid protein particles,[68] as well as that of phospholipase Cg and PKCa in astrocytes.[69] Diacylglycerol (DG) is also generated on this particle.

Thus, cholesterol enrichment of HDL in its assembly reaction is associated with mobilization of cholesterol from the intracellular compartment common to that for cholesterol esterification. Intracellular signaling involving PKC seems required for triggering this process (Figure 1). It is still unclear whether cholesterol is incorporated after apolipoprotein–phospholipid particles are assembled or the lipid compartment for the HDL assembly is enriched in cholesterol by a cholesterol mobilization system(s) discussed above.

Autocrine Reaction for Apolipoprotein to Generate HDL

Major sites for the synthesis of helical apolipoproteins, especially for apoA-I, the main apolipoprotein of HDL, are the liver and intestine. Therefore, these organs are believed to be the major sites of the HDL production as well. In contrast

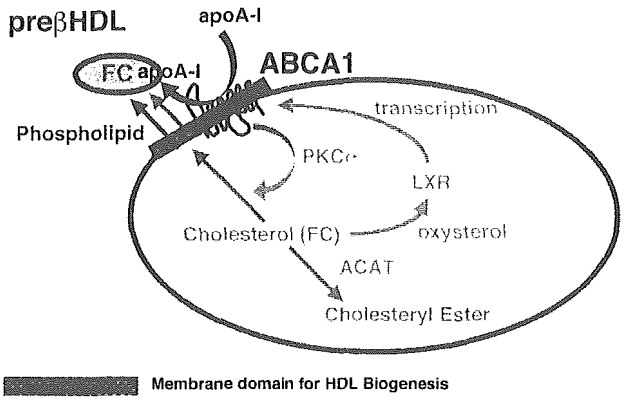

Figure 1. Cellular cholesterol homeostasis and HDL biogenesis. This figure shows roles of the intracellular cholesterol compartment common to the ACAT reaction and the HDL biogenesis. Cholesterol molecules are mobilized from this compartment to a specific membrane domain for the HDL biogenesis being mediated by the action of PKC presumably triggered by the apolioprotein–ABCA1 interaction. HDL is, thus, enriched in cholesterol. Increase of this compartment results in upregulation of ABCA1 perhaps because of the increase of oxysterol that stimulates LXR.

to apolipoprotein B-containing lipoproteins, however, no HDL particles, not even premature HDL, has been identified in the secretary pathways of the cells of these organs, such as the endoplasmic reticulum and the Golgi apparatus. Nevertheless, HDL particles are found in the culture media of the hepatocytes[70,71] or in the perfusate of the liver,[72,73] mostly as a so-called nascent HDL that is largely similar to those generated by the apolipoprotein/ABCA1 reaction. Therefore, it is proposed that HDL is assembled by an autocrine mechanism, such that apoA-I or E are first secreted by the cells and then interact with the cell surface to generate HDL.[47,74] This hypothesis has been more directly supported by using an antibody specific to lipid-free apoA-I, 725-1E2, to inhibit ABCA1-dependent HDL assembly by hepatocytes.[44] Thus, lipid-free apolipoprotein is released from the cells and interacts with cellular ABCA1 for assembly of HDL particles with cellular lipids (Figure 2). Alternatively, apolipoproteins may already interact in part with the membrane somewhere before the secretion through the same mechanism that extracellular apolipoprotein reacts through.[75,76] This view may be consistent with the finding of the abnormal Golgi structure in the hepatocytes of ABCA1 knockout mice[27] and differential generation of HDL with endogenous apoE and exogenous apoA-I by rat astrocytes.[61] Also, the findings discussed above may not exclude the possibility that the extracellular apolipoprotein, whether endogenously or exogenously provided, is internalized, and HDL particles are assembled during the recycling process.[52] Discussed later (Figure 2).

How Does HDL Remove Cell Cholesterol?

When the cells are incubated with HDL particles, cellular cholesterol substantially decreases.[3] This net removal of cholesterol appears with the properties of both nonspecific exchange and apolipoprotein-mediated reaction. LCAT induces the net release of cell cholesterol by the whole HDL particle, even from cells that do not interact with apolipopro-

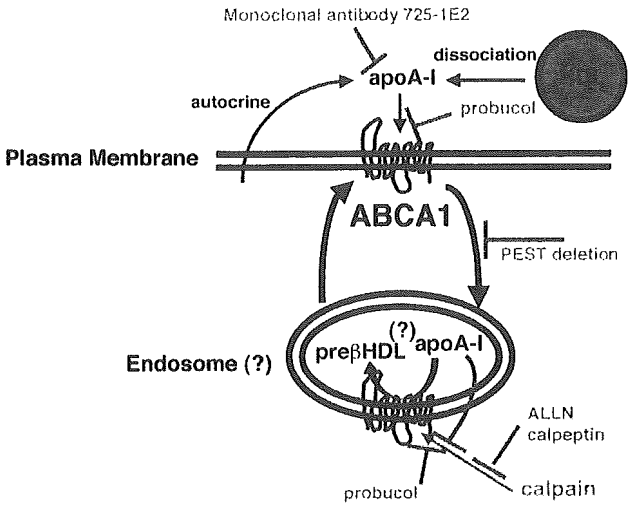

Figure 2. A speculative scheme for the HDL assembly reaction by apolipoprotein and ABCA1. It accommodates the concepts of ABCA1 recycling, roles of free apolipoprotein, the effect of probucol, the results of PEST deletion, and direct and indirect inhibition of the calpain-mediated ABCA1 degradation.

tein,[43,77–79] which is consistent with the former pathway. On the other hand, HDL reduces cell cholesterol[43] and induces various cellular events and reactions, many of which are reproduced by free apolipoproteins, such as removal of intracellular cholesterol,[80] rapid reduction of the ACAT-available cholesterol pool,[58] and PKC activation,[55] representing the characters of the latter pathway.

Helical apolipoproteins are in equilibrium between a lipid-bound form and a dissociated free form in solution. A dissociation constant of apolipoproteins is not known directly for the HDL surface, but the constant measured for the low-density lipoprotein–size lipid particles are all in the order of 10^{-7} M, which may be applicable for the HDL surface.[81,82] Assuming that the dissociation constant of apoA-I for the HDL surface is in this range, and binding capacity of HDL is just enough to accommodate the total plasma apoA-I, a small percent of plasma apoA-I can be lipid-free in the aqueous phase in equilibrium. The apparent Michaelis constant values for the HDL assembly reaction are <1% of plasma apoA-I concentration for most of cells, so that this reaction can be carried out at the maximum velocity in vivo. In addition, cholesteryl ester transfer protein may release helical apolipoproteins from HDL in the presence of free fatty acids.[83–85] Phospholipid transfer protein[86] by itself also releases apolipoprotein from HDL. This may explain the apparent enhancement of the transfer of cellular phospholipid and cholesterol to HDL by phospholipid transfer protein.[87] Apolipoproteins can be transferred from HDL to the cell surface simply because of the higher affinity of free apolipoproteins for the cells than for the lipid surface.[88]

Furthermore, the above-mentioned monoclonal antibody specific for lipid-free apoA-I, 725-1E3, selectively inhibited an ABCA1-dependent part of cell cholesterol release to HDL, and ABCA1-dependent binding of HDL to cells was only for HDL apoprotein but not for HDL lipid.[89] Kinetic analysis of the data indicated that apoA-I has an affinity for HDL as high as that for the cellular surface, and apoA-I could still be transferred from HDL to the cell surface. Therefore, the HDL–cell interaction is represented by apolipoproteins that dissociate from HDL and interact with the cells in their lipid-free form to generate new HDL particles (Figure 2).

It has been indicated that ABCA1 is recycled between the plasma membrane and endosomes, and this may be required for the assembly of HDL.[90,91] If so, ABCA1 interacts with extracellular apolipoproteins and then the complex is internalized to assemble HDL particles during its recycling between the plasma membrane and the endosome before the exocytosis of the HDL (Figure 2).

Regulation of ABCA1 Activity and HDL Assembly

ABCA1 is a rate-limiting step for HDL assembly, and regulation of its function is important for the control of the plasma HDL level.[33] Expression of the ABCA1 gene is regulated primarily by the liver X receptor (LXR)/retinoid X receptor (RXR) system.[92–95] A physiological ligand for LXR is oxysterol, and this ligand may increase in proportion to the cellular cholesterol level. ABCA1 is indeed upregulated by loading cells with cholesterol or inhibiting its intracellular esterification and downregulated by depleting cholesterol (Figure 1).[96–98] Agonists of both receptors have also been shown to upregulate the ABCA1 gene, increase the cellular ABCA1 level, and enhance the release of cellular lipid by apoA-I.[99] Thus, ABCA1 is likely to function for cholesterol homeostasis to reduce cellular cholesterol when it is overloaded.

The ABCA1 gene is upregulated also by other factors. Peroxidase proliferator-activated receptor α agonists and fibric acids increase its transcription in an LXR-dependent manner.[100,101] cAMP strongly induces the ABCA1 gene transcription to increase ABCA1 expression and apoA-I–mediated cell lipid release in certain types of cells, especially in macrophage cell line cells, such as RAW264, THP-1, and J774 cells,[29,30,47,102] seemingly in an LXR/RXR-independent manner. Some calcium channel blockers also induced the ABCA1 gene transcription independent of the LXR/RXR system, resulting in the increase of ABCA1 and apoA-I–mediated lipid release.[103] Thus, pharmacological regulation of the ABCA1 gene transcription directly associates with the level of ABCA1 protein and release of cellular lipid by apolipoprotein. In vivo administration of an LXR/RXR ligand, indeed, results in the increase of plasma HDL.[104]

On the other hand, ABCA1 is rapidly degraded by calpain-mediated proteolysis.[105,106] Helical apolipoproteins protect ABCA1 from this calpain-mediated degradation and thereby increase the ABCA1 level (Figure 2).[105] Phosphorylation of ABCA1 is induced by helical apolipoproteins when it generates HDL, and this is related to its protection from proteolysis.[107] The same results have been demonstrated with synthetic peptides that interact with ABCA1 and generate HDL.[108]

As to the signal to trigger ABCA1 phosphorylation, replenishment of sphingomyelin is suggested to be involved.[107,109] When HDL is assembled with cellular phospholipid, sphingomyelin is removed together with glycerophospholipid (mostly phosphatidylcholine) from the cell membrane. To

replenish sphingomyelin, phosphorylcholin is generated from phosphatidylcholine by phospholipase C and transferred to ceramide. DG is released in these reactions, and PKC is activated by this process. This is a relatively slow reaction and is distinguished from rapid production of DG, activation of PKC, and mobilization of intracellular cholesterol.[69,98] These findings are apparently inconsistent with other observations that the PEST (proline-glutamate-cerine-threonine) sequence in mouse ABCA1 is responsible for its phosphorylation, and apoA-I stimulation of the cells dephosphorylate this site in relation to its stabilization against calpain.[110] In addition, unsaturated free fatty acids increased ABCA1 degradation[111] and its phosphorylation.[112] The reason for the apparent discrepancy between the 2 streams of the evidence is unknown.

Probucol inhibits functions of ABCA1. It does not alter intracellular distribution of ABCA1 or the traffic of cholesterol. It inactivates ABCA1 in the plasma membrane, not only for its function to mediate apolipoprotein binding to the cells and assembly of HDL, but also for its susceptibility to calpain-mediated proteolytic degradation.[113] Accordingly, the cellular ABCA1 level increases by probucol, although it is totally unfunctional (Figure 2).[113] Cyclosporine A inactivates ABCA1 in a very similar manner to that of probucol for its biological functions and susceptibility to calpain in the membrane.[114] Interestingly, the deletion of the PEST sequence caused an increase of ABCA1 by inhibition of its internalization and decrease of its activity for cellular lipid release,[115] in contrast to direct inhibition of calpain that results in the increase of ABCA1 activity. Considering the reports that ABCA1 is recycled between the cell surface and endosomes for the assembly of HDL,[90,91] calpain-mediated degradation of ABCA1 may take place in this process, and phosphorylation of the PEST sequence may be required for this process (Figure 2). Cyclosporine A may also block this process, which leads to the inhibition of the degradation and the HDL assembly,[114] but probucol makes ABCA1 resistant to calpain more directly.[113] Calpain inhibitors do not inhibit internalization and recycle of ABCA1 so that the increase of ABCA1 would result in enhancement of the HDL assembly.[105] A different observation was reported in which probucol prevents ABCA1 from reaching the plasma membrane in different cells in different conditions.[116] The reason for the difference is unknown.

Conclusion

ABCA1 undoubtedly plays an essential role in apolipoprotein-mediated assembly of HDL as its main source. It is, however, still unclear how ABCA1 mediates the interaction of helical apolipoprotein with phospholipid in cell membranes. To maintain cholesterol homeostasis, nonspecific physicochemical cholesterol release is as effective as the apolipoprotein-mediated pathway, both at a cellular level and the whole body. Therefore, Tangier patients may not develop general and massive cholesterol accumulation, because the diffusion-mediated system is preserved.[18] This is the same in the LCAT deficiency patients who lack a driving force for the net cholesterol release by the diffusion-mediated system but not the apolipoprotein-mediated reaction.[117] Thus, the 2 systems back up each other to maintain cellular and body cholesterol homeostasis.[118]

Acknowledgments

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Minireview

ABCA7, a molecule with unknown function

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Abstract **Many ABC proteins are functional in cellular lipid transport in various different manners. ABCA7 is a full-size ABC transporter, the physiological function of which is unknown to date. This is a protein that shows the highest homology known to ABCA1, an essential molecule for producing of plasma high-density lipoprotein (HDL), and in fact it mimics ABCA1 to mediate the production of HDL from cellular lipid when transfected in vitro. It is therefore rational to assume that ABCA7 plays a relevant role in regulating of lipid metabolism. However, the ABCA7 expression profile is distinct from that of ABCA1, with respect to tissue-specific distribution and response to some reagents, presumably because of different transcriptional and posttranscriptional regulation. Potential roles and functions of ABCA7 in lipid homeostasis are discussed, especially in relation to HDL metabolism, based on available publications.**

Keywords: ABCA7; High-density lipoprotein; Apolipoprotein A-I

1. Introduction

ABCA7 is one of the ABC family proteins that is a so-called full-size ABC transporter consisting of two sets of the multiple membrane-spanning domains plus the Walker motifs for the ATP interaction [1]. ABCA7 is a protein having the highest homology known to ABCA1, one of the essential proteins for cholesterol homeostasis. Mammalian ABCA1 plays an important role in cellular and body cholesterol homeostasis, by mediating production of plasma high-density lipoprotein (HDL) with helical apolipoprotein and cellular lipid and thereby regulating cellular cholesterol release [2,3]. Therefore, it is assumed that ABCA7 may also function in lipid or cholesterol metabolism. When ABCA7 was transfected in vitro, it in fact mediates production of HDL upon the interaction of the cells with helical amphiphilic surface-active proteins such as apolipoprotein (apo) A-I, apoA-II and serum amyloid A protein, except that relative release of cholesterol to phospholipid is much lower than the ABCA1-mediated reaction [4–9, Abe-Dohmae et al. manuscript submitted]. However, deficiency of ABCA7 may not markedly alter the plasma HDL level or other lipid/cholesterol metabolism [8] and the tissue-specific expression profile is substantially different from that of ABCA1 [10–12]. Thus, the physiological function of ABCA7 is still essentially unknown. In this article, we intend to review the up-to-date information on ABCA7 and discuss potential functions of this protein based on the available published results.

2. Structure of ABCA7

Human [10], mouse [11], and rat [12] ABCA7 cDNAs have been cloned from macrophages, thymus, and platelets, respectively, and the clones exhibited high homology to other ABC transporters. The cDNAs of ABCA7 encode proteins of 2146 (human) [10], 2159 (mouse) [11], and 2170 (rat) [12] amino acids, respectively. Human ABCA7 protein shows the highest homology known to human ABCA1 (54%) and human ABCA4 (49%) [10]. Interspecies identity of protein sequences of ABCA7 is 79% between human and mouse, less than that of ABCA1 (95%) and ABCA4 (88%) genes [11]. ABCA7 proteins of all the three species contain two sets of ATP-binding cassettes including Walker A and B motifs, which are present in many ATP-utilizing proteins, and a Walker C signature motif, characteristic to the full-size ABC transporters [1]. Interestingly, a cDNA of human ABCA7 has been also cloned from thymus [13] as a gene encoding SS-N, an epitope of an autoantigen related to Sjögren's syndrome.

A splicing variant mRNA (type II mRNA) of human ABCA7 has been detected [5]. The new exon found between exon 5 and 6, which was designated as exon 5B, contained two terminal codons and another translation initiation codon. Thus type II mRNA produces ABCA7 protein containing 28 novel N-terminal amino acids instead of the 1–166 amino acid sequence in full length (type I) ABCA7. Tissue-specific alternative splicing was detected by RT-PCR. Among the tissues examined, expression of type II mRNA was higher than type I mRNA in spleen, thymus, lymph node, and trachea, while type I mRNA was equal to or more than type II mRNA in bone marrow and brain [5]. In this review, type I ABCA7 and type II ABCA7 are designated as ABCA7 and type II ABCA7, respectively.

In Western blotting analysis, protein bands of different molecular weight have been demonstrated in some tissues from mouse [4] and rat [12], suggesting alternative splicing and/or alternative posttranslational modifications.

3. Clinical relevance and gene disruption

No genetic defect was reported on the human ABCA7 gene. A high-density single nucleotide polymorphism (SNP) map of

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