Assembly of High-Density Lipoprotein

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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. 0;0:0-0.)

Key Words: HDL ■ ABCA1 ■ apoA-I ■ apoE ■ cholesterol ■ caveolin ■ membrane ■ cholesterol efflux

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 hormones and bile acids but cannot be converted to energy. Cholesterol is also important as a precursor of steroid membrane proteins, such as those related to signal transduction.

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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. 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 LCAT14,15. 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.14 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, 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 xanthomas38 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 vitro43-44 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-apolipoprotein 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 phospholipid, and it should be in a snap-in manner rather than “gradual growth.” In fact, HDL particles are generated primarily with membrane phospholipid 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 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. Fifty, Fifty-One 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. Fifty-One In contrast, no such rapid and dramatic change takes place when cell cholesterol is removed by nonspecific exchange. Fifty-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, Fifty-Five Fab fragments of anti–apoA-I antibody inhibit the removal of cholesterol from the intracellular pool but not from plasma membrane, Fifty-Six and fibroblasts of Tangier disease patients showed impairment of intracellular cholesterol removal by HDL. Nineteen, Fifty-Seven 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 Fifty-Eight, Fifty-Nine in which apoA-I/A-II heterodimer, presented as a complex with lipids, is responsible for this stimulation. Sixty

Rat astrocytes secrete cholesterol-rich HDL by endogenous apolipoprotein E (apoE) and cholesterol-poor HDL by exogenous apolipoproteins including apoA-I and apoE. Sixty-Four 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. Sixty-Two, Sixty-Three The involvement of caveolin-I, a molecule related to intracellular cholesterol trafficking to a specific membrane domain, such as caveola rich in cholesterol and sphingomyelin, was demonstrated Fourteen-Sixty-Nine in the cholesterol export to HDL and, more specifically, in cholesterol trafficking to the apolipoprotein-mediated HDL generation. Apo-A-I induces rapid translocation of caveolin-I and cholesterol to cytosolic lipid protein particles, Sixty-As well as that of phospholipase Cg and PKCα in astrocytes. Sixty-Nine 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 to apolipoprotein B-containing lipoproteins, however, no HDL particles, not even premature HDL, has been identified in the secretory 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 Seventy-Two, Seventy-Three or in the perfusate of the liver Seven-Two, Seventy-Three 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. Seventy-Four This hypothesis has been more directly supported by using an antibody specific to lipid-free apo-A-I, 725-1E2, to inhibit ABCA1-dependent HDL assembly by hepatocytes. Seventy-Four 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. Seventy-Five, Seventy-Six This view may be consistent with the finding of the abnormal Golgi structure in the hepatocytes of ABCA1 knockout mice Twenty-Two and differential generation of HDL with endogenous apoE and exogenous apoA-I by rat astrocytes. Fifty-One 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. Fifty-Two Discussed later (Figure 2).

How Does HDL Remove Cell Cholesterol?

When the cells are incubated with HDL particles, cellular cholesterol substantially decreases. Three 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 apolipoprotein.
low-density lipoprotein–size lipid particles are all in the order for the HDL surface, but the constant measured for the dissociation constant of apolipoproteins is not known directly bound form and a dissociated free form in solution. A ing the characters of the latter pathway.

available cholesterol pool,58 and PKC activation,55 represent-

intracellular cholesterol,80 rapid reduction of the ACAT-
reproduced by free apolipoproteins, such as removal of 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-

ment of the transfer of cellular phospholipid and cholesterol poproteins from HDL in the presence of free fatty acids.83–85 Cholesteryl ester transfer protein may release helical apoli-

carried out at the maximum velocity in vivo. In addition, 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 apoli-

Phospholipid transfer protein86 by itself also releases apoli-

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).

**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.**

**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. Peroxisome 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 cells29,30,47,102 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.\textsuperscript{69,98} These findings are apparently inconsistent with other observations that the PEST 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.\textsuperscript{110} In addition, unsaturated free fatty acids increased ABCA1 degradation\textsuperscript{111} and its phosphorylation.\textsuperscript{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.\textsuperscript{113} Accordingly, the cellular ABCA1 level increases by probucol, although it is totally unfunctional (Figure 2).\textsuperscript{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.\textsuperscript{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,\textsuperscript{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,\textsuperscript{100,91} 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.\textsuperscript{114} but probucol makes ABCA1 resistant to calpain more directly.\textsuperscript{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.\textsuperscript{105} A different observation was reported in which probucol prevents ABCA1 from reaching the plasma membrane in different cells in different conditions.\textsuperscript{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.\textsuperscript{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.\textsuperscript{117} Thus, the 2 systems back up each other to maintain cellular and body cholesterol homeostasis.\textsuperscript{118}

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References


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7


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