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Commentary

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Putting cholesterol in its place: apoE and reverse cholesterol transport

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To avoid toxic overload of cholesterol in peripheral cells, the reverse cholesterol transport pathway directs excess cholesterol through HDL acceptors to the liver for elimination. In this issue of the *JCI*, a study by Matsuura et al. reveals new features of this pathway, including the importance of the ATP-binding cassette transporter G1 in macrophages and apoE in cholesteryl efflux from cells to cholesterol ester-rich (CE-rich) HDL₂ acceptors (see the related article beginning on page 1435). One proposal for boosting reverse cholesterol transport has been to elevate plasma HDL levels by inhibiting CE transfer protein (CETP), which transfers CE from HDL to lower-density lipoproteins. However, there has been concern that large, CE-rich HDL₂ generated by CETP inhibition might impair reverse cholesterol transport. ApoE uniquely facilitates reverse cholesterol transport by allowing CE-rich core expansion in HDL. In lower species, these large HDLs are not atherogenic. Thus, CETP might not be essential for reverse cholesterol transport in humans, raising hope of using a CETP inhibitor to elevate HDL levels.

The reverse cholesterol transport pathway

While cholesterol is an essential component of mammalian cell membranes, in excess it is toxic to cells. Reverse cholesterol transport is a critical pathway for maintaining cholesterol homeostasis in mammals (1–4). Since the cholesterol ring structure cannot be broken down by cells into carbon dioxide and water, this pathway has evolved to transport excess cholesterol from peripheral cells to the liver for excretion directly into bile or for conversion to bile acids and then excretion.

A number of cellular lipid transporters and receptors and a spectrum of HDL and HDL intermediates participate in this movement of peripheral cholesterol (Figure 1). At one end of the spectrum are lipid-free or lipid-poor apoA-I particles, referred to as pre- β HDL. These particles are secreted by the liver and small intestine or generated from surface material from partially lipolyzed chylomicrons or from HDL₂ in the periphery by the action

of cholesteryl ester (CE) transfer protein (CETP), hepatic lipase, or phospholipid transfer protein (5–9). These HDL precursors are acceptors for unesterified cholesterol and phospholipids transferred by the ATP-binding cassette transporter A1 (ABCA1) on peripheral cells, giving rise to discoidal lipoproteins containing apoA-I (for review, see refs. 2, 10, 11). The acquired unesterified cholesterol is esterified by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT). The CE is then packaged into the hydrophobic core of the discoidal particle, converting it to spherical HDL₃. HDL₃ can continue to accept unesterified cholesterol and phospholipids from the class B, type I scavenger receptor (SR-BI); through continued action of LCAT, the core expands and the particle size increases, forming HDL₂. Although the major apolipoprotein component in HDL₃ and HDL₂ is apoA-I, HDL₂ also contains significant amounts of apoE (12, 13).

The study by Matsuura et al. in this issue of the *JCI* (14) adds ABCG1 present on macrophages to the list of components that contribute to the formation of HDL₂. This is of particular relevance, as monocyte-derived macrophages enter the artery wall, where they scavenge accumulating cholesterol in an attempt to pre-

vent its buildup, leading to an atherosclerotic plaque. Therefore, it is important that cholesterol-loaded macrophages, or foam cells, possess a mechanism to eliminate excess cholesterol before they suffer the toxic effects of this excess.

Differences in reverse cholesterol transport among species

To complete the transport process from HDL₂ to the liver in humans, primates, and a few other species, CE is transferred by CETP to lower-density lipoproteins (Figure 1), which deliver their cholesterol loads through interaction with LDL receptors in the liver. Alternatively, HDL₂ can directly transfer CE to hepatic cells via SR-BIs (15). However, many lower species lack CETP (16) and use HDL as their main plasma cholesterol transporters. In CETP-possessing species, LDLs carry the majority of the plasma cholesterol.

In the absence of CETP, how do these species complete reverse cholesterol transport? A major distinguishing characteristic of their HDL is the presence of a prominent subclass that is enriched in apoE, referred to as HDL₁, HDL_c, or HDL-with apoE (for review, see refs. 12, 17, 18). Since apoE is an effective ligand for LDL receptors, these apoE-enriched HDLs can deliver cholesterol acquired from the periphery to the liver via hepatic LDL receptors (19). CETP-deficient species are particularly resistant to diet-induced elevations in plasma cholesterol levels, demonstrating that they possess an effective reverse cholesterol transport pathway in the absence of CETP. They accomplish this through HDL-with apoE. Their high HDL concentrations and resistance to atherosclerosis are also consistent with the inverse correlation between HDL levels and risk of atherosclerosis observed in humans (1).

Concentrations of HDL-with apoE are low in humans (12, 13) and are thought to represent a minor component of reverse cholesterol transport. However, in subjects with CETP mutations that lead

Nonstandard abbreviations used: CE, cholesteryl ester; CETP, CE transfer protein; LCAT, lecithin:cholesterol acyltransferase.

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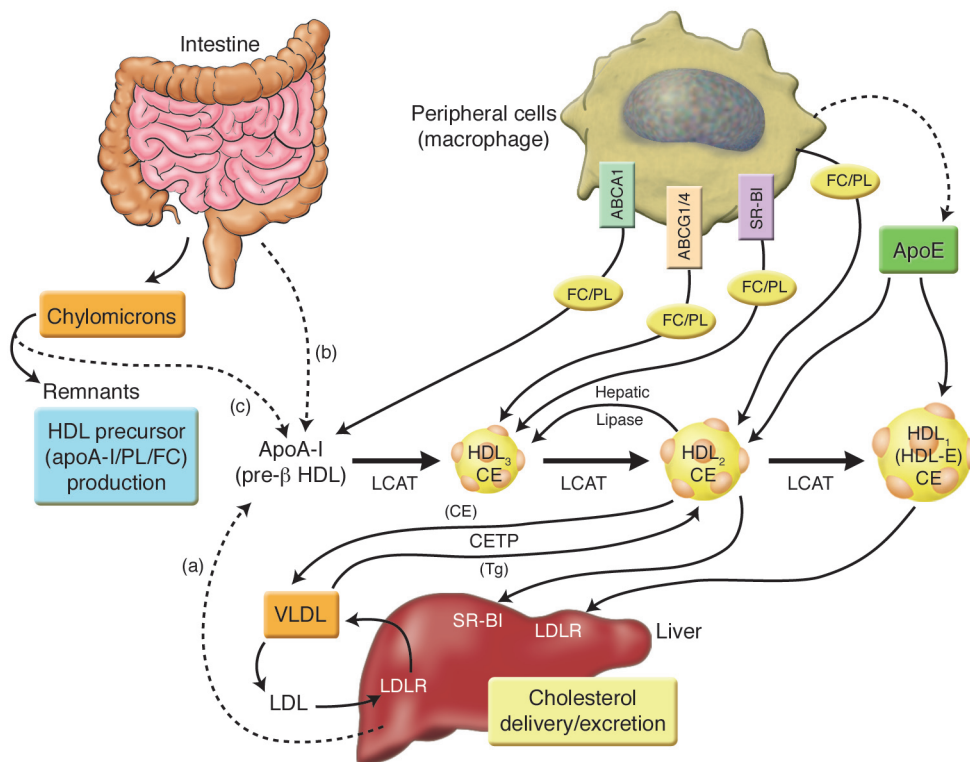


Figure 1
 Role of HDL in the redistribution of lipids from cells with excess cholesterol (e.g., macrophages) to cells requiring cholesterol or to the liver for excretion. HDL precursors (pre-β HDL) are produced by the liver and intestine (apoA-I/PL/FC) or derived from surface material from chylomicrons after lipolysis (a, b, c). Pre-β HDL, HDL₃, and HDL₂ can accept cholesterol for reverse cholesterol transport. ApoE allows HDL₂ particles to expand by enrichment with CE of the core after LCAT converts free cholesterol to CE. Larger HDL-with apoE (HDL₂ or HDL₁) can deliver cholesterol to the liver directly via the LDL receptor. FC, free cholesterol; HDL-E, HDL-with apoE; LDLR, LDL receptor; PL, phospholipid; SR-BI, class B, type I scavenger receptor; Tg, triglyceride.

to CETP deficiency, the HDL-with apoE subclass is markedly increased, resulting in elevated HDL levels (20). Despite the potential benefit of such an antiatherogenic HDL profile in these subjects, there has been a conflict in the literature as to whether CETP deficiency is pro- or anti-atherogenic in humans (21, 22). Thus, it is unclear whether the CETP inhibitors currently in clinical trials would promote or prevent atherosclerosis and coronary heart disease. Matsuura et al. (14) addressed an important aspect of this question and demonstrated that HDL-with apoE in CETP-deficient humans can

accept unesterified cholesterol from macrophages and that LCAT esterified the cholesterol. This resulted in particle size expansion in the presence of apoE, which presumably could deliver this cholesterol directly to the liver.

Importance of apoE for HDL particle expansion

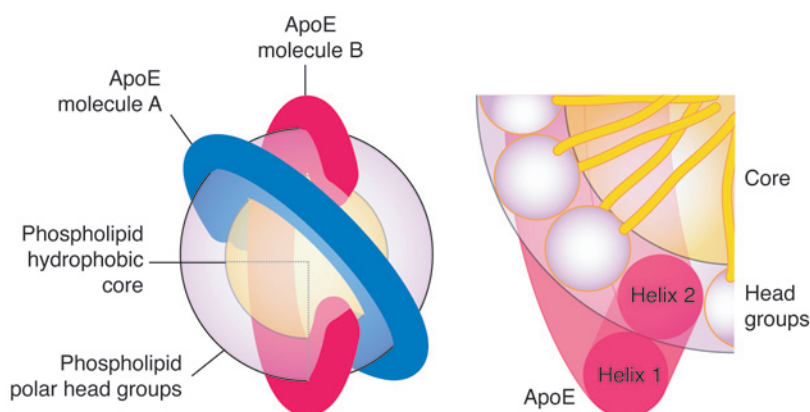
Previously, it was demonstrated that feeding a cholesterol-rich diet to swine resulted in large HDL-with apoE (~180 Å) enriched in CE that approached the size of LDL (~220 Å) (23, 24). Using a combination of calorimetry and small-

angle x-ray scattering, it was shown that, compared with LDL, which contained 2 ordered layers of CE in its core, the relatively smaller swine HDL-with apoE contained a single order layer, accounting for the size difference (25). In both in vivo cholesterol feeding studies and in vitro studies of cholesterol loading, HDL-with apoE enlarged significantly through an increase in the number of ordered layers of CE within the core (140–180 Å particles, 1 layer; 200–220 Å, 2 layers; 240–260 Å, 3 layers) (26, 27).

The importance of apoE in accepting cholesterol from cholesterol-loaded

Figure 2

Based on x-ray crystallographic data, apoE is envisioned to form 2 circular horseshoe-shaped bands around a spherical phospholipid particle. Left: The light yellow core in the center represents the phospholipid hydrophobic core containing the fatty acyl chains; the outer light purple ring represents the phospholipid polar head groups. ApoE primarily interacts with the polar head groups, not the core acyl chains. Thus, apoE on a particle is uniquely capable of facilitating core expansion and accommodating increased CE content following LCAT activity. Right: Cross section of a region of the particle showing the relationship of the 2 apoE helices to the surface phospholipid head groups and core acyl chains.





macrophages and facilitating the expansion of the CE core of HDL in conjunction with the action of LCAT was demonstrated in a series of studies with canine HDL. In one study, canine HDL-with apoE decreased CE formation and accumulation in cholesterol-loaded macrophages, reflecting enhanced cholesterol efflux from the cells; the efficiency of this effect was correlated with protection from atherosclerosis in animal models lacking CETP (28). In an *in vitro* study, when HDL in canine serum was incubated with cholesterol-celite particles or cholesterol-loaded macrophages as sources of cholesterol, the particles increased in size and CE content (26). The results were consistent with esterification of acquired cholesterol by LCAT and size expansion facilitated by apoE. A stepwise increase in the size of the HDL-with apoE was consistent with the addition of 1, 2, and 3 ordered layers of CE in the core. Another study demonstrated an obligatory role for cholesterol and apoE in the expansion of HDL (29). Incubation of apoE-depleted canine HDL in the presence of LCAT and cholesterol-loaded J774 macrophages, which do not synthesize apoE, did not result in significant size expansion of the HDL. However, adding exogenous apoE to the incubation resulted in HDL size expansion, CE accumulation, and enrichment in apoE. In addition, the LDL receptor-binding activity was proportional to the apoE content.

Matsuura et al. (14) extended those previous observations by demonstrating that several components of the reverse cholesterol transport pathway in CETP-deficient species, such as apoE and LCAT, also function in humans.

Potential differences in how apoA-I and apoE bind to lipids

The conclusion from previous studies and the study by Matsuura et al. in this issue (14) is that apoA-I-containing HDL can accommodate only a limited amount of CE in its core, resulting in a limited size expansion, whereas the size and CE content of the HDL can be significantly increased when apoE is present. This raises the question of whether apoE and apoA-I bind differently to lipid. Insight into this possibility comes from a recent low-resolution x-ray crystal model of apoE bound to phospholipid (30). By analogy with apoA-I, where phospholipid complexes are discoidal and the apoA-I

helices are wrapped around the edge of the lipid bilayer in a beltlike manner (31), the expectation was that apoE would bind similarly to a lipid. However, the apoE: phospholipid particles were spheroidal rather than discoidal, and the apoE molecule was folded in half, forming a circular horseshoe 2 helices thick and 1 helix wide and not a complete belt (Figure 2). The hydrophobic face of the amphipathic helices of apoA-I interacts with the fatty acid acyl chains of the phospholipids. In contrast, the polar faces of apoE interact with the polar phospholipid head groups. The hydrophobic faces of apoE are involved in helix:helix packing and thus do not interact with the acyl chains in the core. This different mode of interaction with the surface of the particle may allow apoE to more easily accommodate core expansion of HDL than apoA-I as CEs are formed by LCAT.

Future for CETP inhibitors?

Matsuura et al. (14) have shown that the HDL-with apoE from CETP-deficient humans can effectively accept unesterified cholesterol from macrophages and that apoE, in the presence of LCAT, promotes HDL size expansion. These findings, coupled with the previous *in vitro* studies and demonstration of effective reverse cholesterol transport in CETP-deficient animal models, suggest that CETP inhibitors are likely to be effective in generating an antiatherogenic HDL profile in humans. However, as pointed out by Matsuura et al., the answer awaits completion of ongoing clinical trials.

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