Reverse Cholesterol Transport and Atherosclerosis Regression

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Cholesterol is the major component of atherosclerotic plaque. Cholesterol accumulation within atherosclerotic plaque occurs when cholesterol influx into the arterial wall (from apoB-containing lipoproteins) exceeds cholesterol efflux. Increased influx of cholesterol into the arterial wall is accompanied by an increased influx of monocytes/macrophages, which take up oxidized and aggregated LDL and store the cholesterol as esters. Whereas parenchymal cells maintain cholesterol balance by downregulating de novo cholesterol synthesis and LDL receptor expression, macrophages continue to take up cholesterol from apoB-containing lipoproteins via pathways that are not subject to sterol-mediated feedback control. Current strategies to reduce coronary heart disease (CHD) are aimed primarily at reducing the influx of cholesterol into the arterial wall by lowering plasma LDL cholesterol concentrations. Aggressive lowering of plasma LDL levels reduces mortality from CHD, but protection is not complete, and in a significant proportion of patients, plasma LDL concentrations cannot be lowered to a level that would be predicted to halt the progression of disease. As a consequence, there is great interest in strategies aimed at enhancing cholesterol efflux from the arterial wall and promoting its transport to the liver for excretion.

See p 594

Cholesterol that is synthesized in extrahepatic tissues or acquired from lipoproteins is returned to the liver for excretion in a process called reverse cholesterol transport. The initial step in reverse cholesterol transport is thought to be efflux of cholesterol from cell membranes to acceptor particles in the interstitial fluid. Two models have been proposed with regard to the movement of cholesterol from plasma membrane to acceptor particles. In the first, the aqueous diffusion model, cholesterol molecules spontaneously desorb from cell membranes and are then incorporated into acceptor particles after traversing the intervening aqueous space by diffusion. Phospholipid vesicles, phospholipid/albumin complexes, and triglyceride/phospholipid emulsions efficiently remove cholesterol from cells via this mechanism, which does not require interaction with specific cell receptors. The second model involves the interaction of HDL (presumably via apoA-I) with cell surface binding sites, which induces an intracellular signal leading to translocation of cholesterol from intracellular sites to the plasma membrane. This mechanism has been demonstrated mainly in cholesterol-loaded cells and was first suggested by studies in cholesterol-loaded peritoneal macrophages, which showed that HDL$_3$, but not phospholipid liposomes, stimulated the hydrolysis and secretion of stored cholesteryl esters. The physiological acceptor for cholesterol in vivo appears to be nascent HDL particles, which are discoidal pre-β-migrating complexes of phospholipid and apoA-I (other amphipathic apoproteins, such as apoE or apoA-IV, may also be present). These nascent HDL particles are secreted by liver and small bowel and also formed during the metabolism of triglyceride-rich lipoproteins from excess surface material (phospholipid and soluble apoproteins). Cholesterol that is transferred to nascent HDL particles is esterified by lecithin-cholesterol acyl transferase (LCAT) to cholesteryl esters, which by virtue of their hydrophobicity, move into the core of the HDL particles. In this way, nascent HDL is converted to the α-migrating spherical HDL found in plasma.

HDL cholesteryl esters are cleared from plasma through several pathways. In the presence of cholesteryl ester transfer protein (CETP), a portion of the HDL cholesteryl ester is transferred to lower-density lipoproteins (in exchange for triglyceride) and ultimately returned to the liver via the LDL receptor pathway. In addition, HDL cholesteryl esters are delivered to the liver via a nonendocytotic process in which cholesteryl ester is selectively taken up by the liver, resulting in an HDL particle of reduced size and cholesteryl ester content. The liver accounts for 75% of total HDL cholesteryl ester turnover, and uptake occurs via a high-affinity transport mechanism that is saturated at normal plasma HDL concentrations. The final step in the reverse cholesterol transport pathway is excretion of cholesterol from the liver into bile, either directly or after conversion to bile salts.

Individual steps in the reverse cholesterol transport pathway have been studied extensively; however, little is known about what regulates cholesterol flux through the entire pathway. Although there is clearly net movement of cholesterol from extrahepatic tissues to the liver and into bile, there is no way to directly quantify this flux in vivo. An approach that has been used in animal models is to quantify the rate of cholesterol acquisition in all extrahepatic tissues (from de novo synthesis, LDL, and HDL) as a measure of reverse cholesterol transport because, in a steady state, the rate of cholesterol acquisition by the extrahepatic tissues equals the rate of cholesterol return to the liver for excretion (with the exception of cholesterol that is converted into steroid hor-
mones or lost when cells are sloughed from the skin or gastrointestinal tract). This approach has shown that plasma HDL cholesterol concentrations can be varied over a wide range with no change in the rate of reverse cholesterol transport.11

Given the key role that HDL plays in reverse cholesterol transport and epidemiological studies showing an inverse relationship between plasma HDL cholesterol concentrations and the risk of clinical CHD, it was anticipated that raising plasma HDL levels might protect against atherosclerosis. Indeed, repeated injections of HDL12 or purified apoA-I13 were shown to be protective in cholesterol-fed rabbits, and repeated injections of apoA-I/phospholipid complexes inhibited progression of atherosclerosis in cholesterol-fed apoE-deficient mice.14 Overexpression of human apoA-I reduced the progression of atherosclerotic lesions in C57BL/6 mice fed an atherogenic diet15 and in apoE-deficient mice fed standard chow.16 Although it is possible that these interventions increased reverse cholesterol transport, there is no evidence that this in fact occurred. The protective effect of HDL could have resulted from antioxidant effects or other mechanisms unrelated to reverse cholesterol transport.

Notably, intravenous administration of phospholipid liposomes was shown to induce regression of preexisting atherosclerosis nearly 45 years ago.17 The intravenous injection of phospholipid liposomes results in net cholesterol movement from tissues into plasma, as evidenced by a rapid and dose-dependent increase in plasma unesterified cholesterol concentrations. The metabolic fate of tissue cholesterol that has been mobilized into plasma depends mainly on the size of the infused liposomes.18 The majority of large multilamellar vesicles are cleared by the reticuloendothelial system (mainly in liver and spleen). Small unilamellar vesicles are apparently cleared by parenchymal cells in the liver; however, at least in the rabbit, the liver does not excrete this cholesterol into bile. Rather, there is an increase secretion of cholesterol into plasma, suppression of hepatic LDL receptor expression, and a 4-fold increase in plasma LDL concentrations.19

In this issue of Circulation, Eriksson et al19 examined the effect of proapoA-I/phospholipid complexes on the final step in the reverse cholesterol transport pathway, namely, fecal sterol excretion. Discoidal complexes of proapoA-I (or apoA-I) and phospholipid are highly effective at mobilizing cholesterol from normal and cholesterol-loaded cells and closely resemble the nascent HDL particles that are formed in vivo. These complexes are the preferred substrate for LCAT, which makes it likely that cholesterol that is transferred from tissues to these particles will be esterified, leading to the formation of α-migrating spherical HDL. Remarkably, a single intravenous injection of reconstituted proapoA-I/phospholipid complexes resulted in a 30% increase in fecal bile salt excretion and a 39% increase in neutral sterol excretion in 4 patients with heterozygous familial hypercholesterolemia. This translated into an extra 5 g of cholesterol excreted from the body during the 9 days after injection of the proapoA-I/phospholipid complexes.

This interesting article raises a number of important questions. First, by what mechanism does an injection of proapoA-I/phospholipid complexes induce fecal sterol excretion? One possibility is that administration of proapoA-I/phospholipid complexes increased the flux of cholesterol from extrahepatic tissues to the liver and that the liver responded by increasing bile salt synthesis and biliary cholesterol output. However, although cholesterol was clearly mobilized from tissues into plasma—as evidenced by the increase in plasma HDL cholesterol concentrations—the increase in plasma HDL cholesterol levels was transient and very small relative to the increase in fecal sterol excretion. Furthermore, there was no relation between the increase in HDL cholesterol levels and the increase in fecal sterol excretion among the 4 patients that were studied. If apoA-I/phospholipid complexes caused the movement of cholesterol from tissues into plasma, the question that follows is, where did the cholesterol come from? Most extrahepatic tissues contain very little stored cholesteryl ester; as a consequence, loss of cholesterol from these tissues will be balanced primarily by an increase in de novo cholesterol synthesis. Conversely, efflux of cholesterol from the plasma membrane of foam cells in the arterial wall should result in the mobilization of stored cholesteryl esters. Intravenously administered apoA-I/phospholipid complexes would probably induce cholesterol efflux from all cells to which they are exposed. It is therefore surprising that Eriksson et al found no evidence for an increase in endogenous cholesterol synthesis (based on a failure to detect an increase in circulating cholesterol precursors in plasma) after the injection of apoA-I/phospholipid complexes.19 In contrast, in animal models (mice and hamsters), in which cholesterol synthesis can be quantified directly by use of tritiated water, the increase in plasma cholesterol concentration that follows an infusion of apoA-I/phospholipid complexes is accompanied by an increase in cholesterol synthesis in nearly all tissues, including the liver, indicating that these complexes mobilize cholesterol from the liver as well as the extrahepatic tissues (D.K. Spady and R.S. Meidell, unpublished observation). Nevertheless, it may still be possible to achieve net cholesterol movement from extrahepatic tissues to the liver, because cholesterol that is transferred to apoA-I/phospholipid complexes will be esterified by LCAT, leading to the generation of α-migrating spherical HDL, and it is known that most HDL cholesteryl ester is delivered to the liver either directly or after transfer to lower-density lipoproteins.

If the rate of reverse cholesterol transport is increased, what will be the response of the liver? It may serve no useful purpose to increase the flux of cholesterol from extrahepatic tissues to the liver if the cholesterol simply accumulates in the liver, suppresses hepatic LDL receptor expression, and ultimately is secreted back into plasma as apoB-containing lipoproteins. In the rat, infusion of a hypercholesterolemic LDL/HDL fraction resulted in a marked increase in liver cholesteryl ester levels and a 5-fold increase in VLDL secretion but no increase in biliary cholesterol secretion.20 Thus, it may not be safe to assume that increasing the flux of cholesterol to the liver will be entirely benign.

Influx of cholesterol into the arterial wall can now be controlled by aggressively lowering the plasma concentration of apoB-containing lipoproteins. Although disease progression is usually slowed or halted by aggressive lipid lowering,
regression of preexisting lesions is relatively uncommon. Understanding how to increase the efflux of cholesterol from foam cells within the arterial wall and delivering this cholesterol to the liver for excretion may be key to achieving timely regression of atherosclerotic lesions. Intravenous administration of apoA-I/phospholipid complexes should enhance the initial step in the reverse cholesterol transport pathway (efflux of cholesterol to nascent HDL). If this is the rate-limiting step, then the flux of cholesterol through the entire reverse cholesterol transport pathway may be increased. There is no reason to think that apoA-I/phospholipid complexes are specific for foam cells. Consequently, if reverse cholesterol transport is enhanced, most of the cholesterol will probably come from normal tissues that will increase de novo synthesis to maintain cholesterol balance. Nevertheless, to the extent that net cholesterol movement from extracellular tissues to the liver is increased, it is reasonable to expect that some of this cholesterol will come from foam cells in the arterial wall. Small, lipid-rich plaques, which are most likely to rupture and cause myocardial infarction, may be most amenable to regression. Eventually, up to 30% of plaque cholesterol may be in the form of extracellular crystalline cholesterol. Although more resistant to mobilization, even crystalline cholesterol can be taken up by macrophages21 and—if after degradation and esterification—would be available for efflux from the plaque if reverse cholesterol transport could be accelerated. Although many questions remain, enhancing cholesterol efflux from the arterial wall is an attractive approach that would complement current strategies for reducing cholesterol influx into the arterial wall. By a combination of these approaches, it may be possible to achieve substantial and rapid regression of atherosclerotic lesions. The study by Eriksson et al emphasizes the need for a better understanding of how cholesterol flux through the reverse cholesterol transport pathway is regulated in vivo.

References
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