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Shifting gears: liver SR-BI drives reverse cholesterol transport in macrophages

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Commentary

Cholesterol efflux from macrophages, the first step in reverse cholesterol transport (RCT), is assumed to play a critical role in the pathogenesis of atherosclerosis. However, in vivo proof supporting this hypothesis is lacking, due to difficulties in determining the activity of this first step in RCT. In this issue of the *JCI*, Zhang et al. apply their recently developed method for measuring RCT in vivo to estimate RCT in mouse models with varying levels of HDL turnover. A surprisingly efficient clearance of cholesterol to feces is observed in mice overexpressing hepatic scavenger receptor class B type I (SR-BI), whereas in *SR-BI*–knockout mice, cholesterol clearance is diminished. The study demonstrates that hepatic SR-BI is a positive regulator of macrophage RCT in vivo.

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Shifting gears: liver SR-BI drives reverse cholesterol transport in macrophages

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Cholesterol efflux from macrophages, the first step in reverse cholesterol transport (RCT), is assumed to play a critical role in the pathogenesis of atherosclerosis. However, in vivo proof supporting this hypothesis is lacking, due to difficulties in determining the activity of this first step in RCT. In this issue of the *JCI*, Zhang et al. apply their recently developed method for measuring RCT in vivo to estimate RCT in mouse models with varying levels of HDL turnover. A surprisingly efficient clearance of cholesterol to feces is observed in mice overexpressing hepatic scavenger receptor class B type I (SR-BI), whereas in *SR-BI*–knockout mice, cholesterol clearance is diminished (see the related article beginning on page 2870). The study demonstrates that hepatic SR-BI is a positive regulator of macrophage RCT in vivo.

The transport of excess cholesterol from the periphery into the liver and bile, followed by excretion in the feces, is defined as reverse cholesterol transport (RCT) (Figure 1). Since the original definition of RCT by Glomset and Norum in 1973 (1), this pathway has become increasingly popular as a target for therapeutic strategies aimed at achieving the regression of atherosclerosis. Theoretically, a lipid-laden macrophage can release its contents by activation of efflux pathways. HDL is considered to be the primary cholesterol carrier in RCT, but conclusive evidence for this contention has been lacking until now. It is not an easy task to estimate net cholesterol flux from peripheral tissues to feces. Neutral sterols found in feces are derived from several sources. The major source is the liver, where the bulk of body cholesterol and bile salts are synthesized. After their secretion from the liver in bile, both components are secreted into the intestine, where up to 95% of bile salts and 30–60% of cholesterol is reabsorbed. The nonabsorbed cholesterol partly undergoes bacte-

Nonstandard abbreviations used: RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type I.

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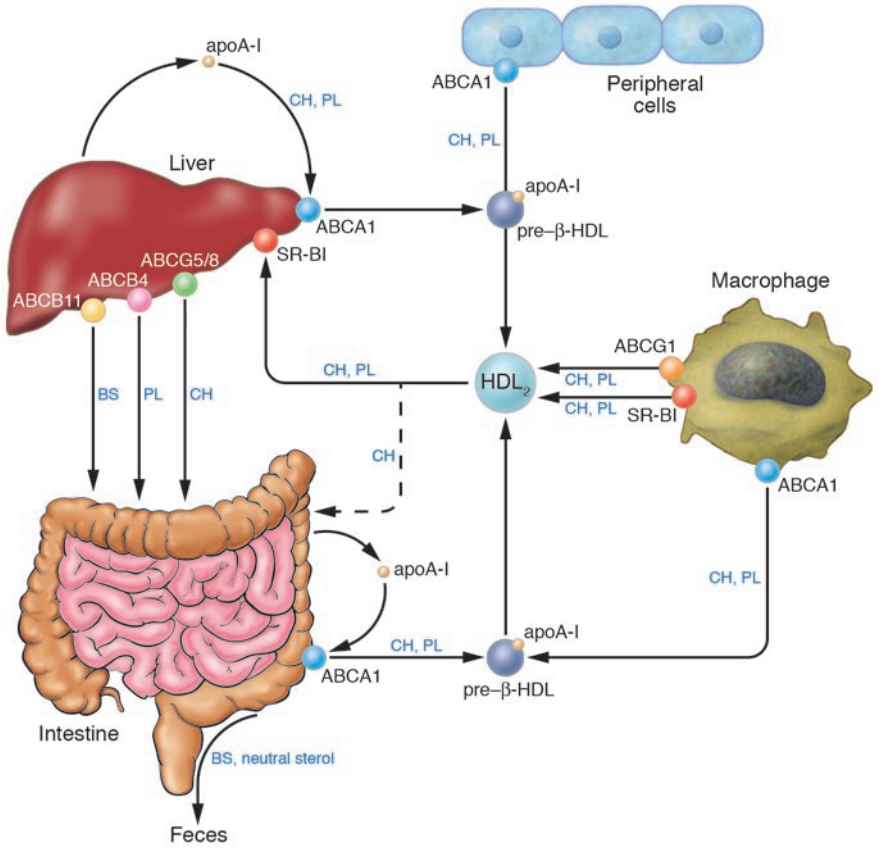

Figure 1
Schematic overview of the major pathways involved in RCT from peripheral tissue and macrophages/foam cells. apoA-I is secreted by liver and intestine and loaded with cholesterol (CH) and phospholipids (PL) by ABCA1. The thus formed pre-β-HDL picks up cholesterol and phospholipid from ABCA1 in macrophages and peripheral cells and is converted to HDL_2. HDL_2 can be further loaded with cholesterol by ABCG1, and possibly SR-BI, in macrophages and delivers in turn its cargo to SR-BI in the liver. Zhang et al. (12), via development of a surrogate method to monitor foam cell cholesterol efflux in mice, have now shown that hepatic SR-BI is a positive regulator of macrophage RCT in vivo. Subsequently, cholesterol can be secreted into the bile either in the free form or after conversion as bile salt (BS). After transport via the bile into the intestine, cholesterol and bile salts are reabsorbed or excreted in the feces.

rial conversion to coprostanol and other neutral sterol metabolites and is excreted in feces together with cholesterol derived from shedded enterocytes and cholesterol that enters the intestinal lumen via direct transintestinal secretion from blood (2). The dedicated contribution of cholesterol from the periphery to total fecal neutral sterol output may be relatively small, and cholesterol derived from macrophages is only part of this peripheral flow.

Regulation of cholesterol efflux from macrophages

An imbalance in the pathways responsible for cellular cholesterol influx and efflux causes the conversion of a macrophage into a foam cell. Influx of cholesterol into macrophages may occur via a number of independent pathways; receptor-mediated endocytosis of modified LDL, mediated by scavenger receptor class A or CD36 serves as the main pathway (3). Uptake of cellular debris may also be an important source of cholesterol. Whereas cholesterol influx mainly follows the endosomal/lysosomal route, cholesterol is effluxed from macrophages in its free form by the concerted action of several parallel pathways, most of them involving the activity of primary active ATP-binding cassette transporters. The ABCA1 and ABCG1 transporters may be involved in the regulation of cholesterol efflux (reviewed in ref. 4). In addition, the HDL scavenger receptor class B type I (SR-BI) may play a role in macrophage efflux, depending on the free energy gradient of cholesterol. ABCA1 has the highest affinity for free apoA-I and pre–β-HDL, whereas ABCG1 and SR-BI probably interact primarily with more mature HDL (4) (Figure 1). In all of these steps, the different forms of HDL play a pivotal role. Consequently, it has long been thought that plasma HDL levels accurately reflect the rate of RCT. Since many epidemiological studies have shown a strong inverse relationship between cardiovascular disease risk and HDL levels, this seemed a plausible paradigm. Particularly elegant studies by Dietschy and colleagues (5–7) have challenged this concept. Jolley, Dietschy, et al. (6) could not discern any effect on cholesterol homeostasis in *Apoa1*-null mice with very low HDL levels. Similar results were reported by our group in experiments with *Abca1*-null mice, in which HDL is almost absent (8). Alam et al. (9) upregulated the expression of proteins that mediate individual steps believed to be involved in HDL trafficking pathways in normolipidemic mice and did not find any effect on RCT. In humans, 2 studies have demonstrated a significant effect of apoA-I or reconstituted HDL infusions on neutral sterol output (10, 11). The major pitfall in all of these studies is the lack of differentiation between the different sources contributing to fecal neutral sterol output. As we have discussed above, cholesterol efflux from foam cells — the most relevant step in RCT with respect to atherosclerosis — may be only a minor contributor to total RCT. All efforts to visualize HDL-mediated regulation of macrophage cholesterol efflux may have failed because of the inability to measure this minor flux.

A surrogate method to determine macrophage cholesterol efflux

In this issue of the *JCI*, Zhang et al. attempt to circumvent this problem by using a surrogate method to monitor cholesterol efflux from macrophages (12). For this purpose, mouse J774 cells were labeled in vitro with [^{3}H]cholesterol and loaded with lipid by incubation with acetylated LDL. Subsequently, the cells were injected into the peritoneum of mice, and RCT was measured by studying the appearance of the label in plasma, liver, and feces. In an earlier study, the authors showed that most of the tritiated cholesterol was esterified in J774 cells, and after 24 hours, a significant amount appeared in the feces, in the form of both bile salts and neutral sterols (13). Clearly, J774 cells are not equivalent to macrophages/foam cells present in the

vessel wall or atherosclerotic lesions, and the results of the study have to be considered in light of this difference. Zhang et al. did not measure specific activities of the different cholesterol pools. This impedes an estimation of cholesterol mass transfer in the different experiments. However, the different mouse models employed in the study do allow some speculation with respect to the preferred metabolic routes involved in the handling of effluxed cholesterol. How cholesterol is transported from the peritoneal cavity to the blood is not clear, but once in plasma, the tritiated cholesterol equilibrates with plasma cholesterol. In wild-type mice, about 2% of the injected label appeared in the feces within the first 24 hours. This is a substantial amount in view of the fact that only 4% of the label was present in plasma. Overexpression of SR-BI in the liver was observed to significantly increase the clearance rate, particularly in the presence of apoA-I overexpression. These findings are very suggestive of an important role for HDL in macrophage cholesterol efflux. Changes in specific activity of plasma cholesterol in the different mouse models may, however, have confounded these apparently clear results. For instance, overexpression of SR-BI induced a 6-fold decrease in total serum cholesterol, whereas the percentage of [^{3}H]cholesterol decreased less than 3-fold and thus invoked an increase in plasma-specific activity (12). This may explain at least part of the observed increase in fecal output of tritiated cholesterol without an increase in net fecal output. Alam et al. (9) used similar mouse models to investigate the influence of variation of processes involved in RCT on fecal sterol output. In mouse models of both SR-BI and apoA-I overexpression, no effect on fecal sterol output was observed, despite similar variations observed in plasma cholesterol levels. Alam et al. (9) measured total fecal excretion and could not discriminate between cholesterol derived from the periphery and that derived from macrophages. Unfortunately, Zhang et al. did not determine total neutral sterol secretion in their experiments, impeding a direct comparison with the study by Alam et al. (9).

Conclusions

A method to assess the rate of cholesterol efflux from foam cells is desperately needed to be able to define the importance of this step in the progression and possibly the regression of atherosclerosis. An initial step toward this goal has been made by Zhang et al. (12). Clearly this method requires further evaluation, but it may be a first step in the development of a surrogate assay to determine RCT in humans.

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