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 1 (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.

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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 ApoA-I-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 [3H]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 triglycerated 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 intestine, cholesterol and bile salts are reabsorbed or excreted in the feces.

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 HDL₂. HDL₂ 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.

**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

**commentaries**
vessel wall or atherosclerotic lesions, and the results of the study have to be consid-
ered 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 trans-
erfer 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 cho-
lesterol. How cholesterol is transported
from the peritoneal cavity to the blood is
not clear, but once in plasma, the tritiated
cholesterol equilibrates with plasma cho-
lesterol. 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. Overex-
pression of SR-BI in the liver was observed to
significantly increase the clearance rate,
particularly in the presence of apoA-I over-
expression. These findings are very sug-
uggestive 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 [$H]cholesterol decreased less than 3-
fold and thus invoked an increase in plas-
ma-specific activity (12). This may explain
at least part of the observed increase in
fecal output of tritiated cholesterol with-
out an increase in net fecal output. Alam et
al. (9) used similar mouse models to inves-
tigate the influence of variation of process-
es 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 varia-
tions observed in plasma cholesterol levels.
Alam et al. (9) measured total fecal excre-
tion and could not discriminate between
cholesterol derived from the periphery and
that derived from macrophages. Unfortu-
nately, Zhang et al. did not determine total
neutral sterol secretion in their experi-
ments, impeding a direct comparison with
the study by Alam et al. (9).

Conclusions
A method to assess the rate of cholesterol
eflux 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 fur-
ther evaluation, but it may be a first step
in the development of a surrogate assay to
determine RCT in humans.

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