

The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis

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Abstract Reverse cholesterol transport (RCT) is a term used to describe the efflux of excess cellular cholesterol from peripheral tissues and its return to the liver for excretion in the bile and ultimately the feces. It is believed to be a critical mechanism by which HDL exert a protective effect on the development of atherosclerosis. In this paradigm, cholesterol is effluxed from arterial macrophages to extracellular HDL-based acceptors through the action of transporters such as ABCA1 and ABCG1. After efflux to HDL, cholesterol may be esterified in the plasma by the enzyme lecithin:cholesterol acyltransferase and is ultimately transported from HDL to the liver, either directly via the scavenger receptor BI or after transfer to apolipoprotein B-containing lipoproteins by the cholesteryl ester transfer protein. Methods for assessing the integrated rate of macrophage RCT in animals have provided insights into the molecular regulation of the process and suggest that the dynamic rate of macrophage RCT is more strongly associated with atherosclerosis than the steady-state plasma concentration of HDL cholesterol. **■** Promotion of macrophage RCT is a potential therapeutic approach to preventing or regressing atherosclerotic vascular disease, but robust measures of RCT in humans will be needed in order to confidently advance RCT-promoting therapies in clinical development.—Rader, D. J., E. T. Alexander, G. L. Weibel, J. Billheimer, and G. H. Rothblat. **The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis.** *J. Lipid Res.* 2009. 50: S189–S194.

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Reverse cholesterol transport (RCT), originally proposed by Glomset (1), is currently understood as the physiologic process by which cholesterol in peripheral tissues is transported by HDL to the liver for excretion in the bile and feces (Fig. 1). Most peripheral cells and tissues (except those in steroidogenic organs) cannot catabolize cholesterol

and can only dispose of it by effluxing it to extracellular acceptors such as HDL. The primary biomedical interest in RCT has been with regard to atherosclerosis; it has long been believed that RCT is the major mechanism by which HDL protects against atherosclerotic cardiovascular disease. Cholesterol-loaded macrophage “foam cells” are the cellular hallmark of the atherosclerotic lesion, and cholesterol efflux from intimal macrophage foam cells may protect against the progression and complications of atherosclerotic vascular disease. Whether the subsequent fate of cholesterol that has been effluxed from macrophages is relevant to atherogenesis remains the topic of debate. Nevertheless, in order to maintain whole-body cholesterol homeostasis, peripheral cholesterol must be returned to the liver and excreted in the bile and feces, completing the RCT pathway. Methods have been developed in animals to assess integrated RCT from macrophages to liver and feces; methods in humans to assess RCT are in the process of development and validation. Promotion of macrophage RCT could be an effective therapeutic strategy for reducing risk associated with atherosclerotic vascular disease (2).

MACROPHAGE CHOLESTEROL EFFLUX IS REGULATED BY SPECIFIC CELLULAR TRANSPORTERS AND INFLUENCED BY THE NATURE AND QUANTITY OF EXTRACELLULAR HDL-BASED ACCEPTORS

The maintenance of optimal cellular cholesterol concentrations is essential for proper cell function and viability, and excess cholesterol is toxic to cells. Macrophages can protect against cholesterol toxicity by converting free

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; HDL-C, HDL cholesterol; LCAT, lecithin:cholesterol acyltransferase; LXR, liver X receptor; RCT, reverse cholesterol transport; PPAR, peroxisome proliferator-activated receptor; SR-BI, scavenger receptor class B type I.

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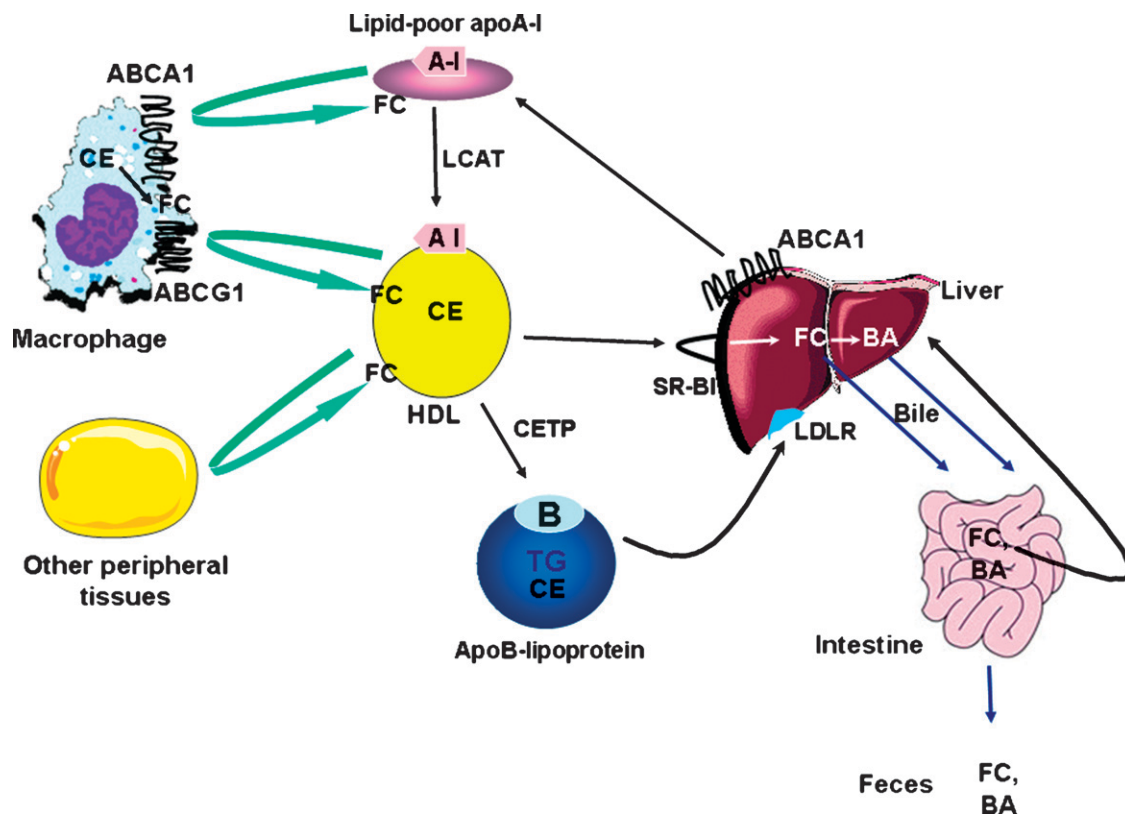


Fig. 1. Reverse cholesterol transport. The liver secretes lipid-poor apoA-I which quickly acquires cholesterol via the hepatocyte ABCA1 transporter. Lipid-poor apoA-I also promotes the efflux of cholesterol from macrophages via ABCA1. Mature HDL is formed by the action of LCAT and promotes cholesterol efflux from macrophages via the ABCG1 transporter, as well as from other peripheral tissues by processes not fully defined. Mature HDL can transfer its cholesterol to the liver directly via SR-BI or indirectly via CETP-mediated transfer to apoB-containing lipoproteins, with subsequent uptake by the liver via the LDL receptor. Hepatic cholesterol can be excreted into the bile directly as cholesterol or after conversion to bile acids and unless reabsorbed by the intestine is ultimately excreted in the feces.

cholesterol to cholesteryl ester or by effluxing cholesterol to extracellular acceptors. The rate of cellular cholesterol efflux is dependent on the cholesterol status of the cell, the level of expression of cholesterol transporters, and the composition and concentration of extracellular cholesterol acceptors, generally HDL or specific subfractions of HDL. The most abundant protein in HDL is apolipoprotein (apo) A-I, which in a lipid-poor form is one type of acceptor; mature HDL particles also serve as acceptors of cellular cholesterol efflux (Fig. 1).

A variety of specific pathways of cholesterol efflux have been defined, including: 1) efflux to lipid-free apolipoproteins, particularly apoA-I, mediated by ABCA1; 2) efflux to mature HDL particles mediated by ABCG1; and 3) efflux to mature HDL particles by other pathways, including possibly scavenger receptor class B type I (SR-BI) as well as passive diffusion. ABCA1- and ABCG1-mediated flux are unidirectional, leading to net removal of cell cholesterol. Because these efflux pathways utilize different types of HDL acceptor particles, the relative levels of acceptor particles play an important role in determining the efficiency of cholesterol efflux when cells are exposed to serum or isolated total HDL.

Loss-of-function mutations in both alleles of ABCA1 in humans (Tangier disease) is characterized by severe HDL

deficiency and cholesterol accumulation in peripheral tissue macrophages, a phenotype also seen in knockout mice lacking ABCA1. Macrophages deficient in ABCA1 expression have significantly impaired cholesterol efflux to lipid-poor apoA-I in vitro. Consistent with this observation, mice that were transplanted with bone marrow from ABCA1 knockout mice have increased atherosclerotic lesion development while maintaining a normal plasma HDL-cholesterol (HDL-C) level (3), whereas mice that were transplanted with bone marrow from ABCA1-overexpressing mice have reduced atherosclerosis (4). Consistent with these findings, ABCA1-deficient macrophages demonstrate significantly reduced RCT in vivo (5).

ABCG1 facilitates cholesterol efflux to mature HDL but not to lipid-poor apoA-I (6, 7). Mice that are deficient in ABCG1 have lipid accumulation in macrophages within multiple tissues when they are fed a high-fat, high-cholesterol diet (7). Macrophages lacking ABCG1 expression have impaired cholesterol efflux to mature HDL ex vivo and demonstrate significantly reduced RCT in vivo (5). Paradoxically, macrophage deficiency of ABCG1 is associated with decreased atherosclerosis (8, 9), possibly due to compensatory upregulation of macrophage ABCA1 and apoE (9) or increased susceptibility of ABCG1-deficient macrophages to oxidized LDL-induced apoptosis (8).

ABCA1 and ABCG1 act in a cooperative fashion to promote macrophage cellular cholesterol efflux. For example, ABCA1 can lipidate lipid-poor apoA-I to generate nascent particles, which can then serve as substrates for ABCG1-mediated cholesterol efflux (10). Knockdown of both ABCA1 and ABCG1 in macrophage reduced cholesterol efflux *ex vivo* and RCT *in vivo* to a greater extent than loss of function of either transporter alone (5). Consistent with this, transplantation of ABCA1/ABCG1 double knockout bone marrow into atherosclerosis-prone mice resulted in substantially greater atherosclerosis than bone marrow from either single knockout alone (11).

Macrophage expression of ABCA1 and ABCG1 is regulated by the nuclear receptor liver X receptors α and β (LXR α and LXR β), which act as heterodimers with their partner, the retinoid X receptor. Synthetic LXR agonists upregulate ABCA1 and ABCG1 transcription in macrophages and promote increased cholesterol efflux to both lipid-poor apo A-I and mature HDL *ex vivo* and macrophage RCT *in vivo* (12). Synthetic LXR agonists have been shown to inhibit atherosclerosis progression (13) and even promote atherosclerosis regression (14) in mice despite having little effect on plasma HDL-C levels. Notably, both peroxisome proliferator-activated receptor (PPAR)- α and PPAR γ agonists have been reported to promote macrophage cholesterol efflux *in vitro* through upregulation of ABCA1 (15, 16), and both classes were also shown to limit macrophage foam cell formation *in vivo* and inhibit atherosclerosis (17).

The role of macrophage SR-BI in macrophage cholesterol efflux and protection from atherosclerosis remains uncertain. Certainly SR-BI can promote cholesterol efflux to mature HDL *in vitro*, although it can also promote uptake of HDL-C and therefore is a bidirectional transporter (18). In mice, there was no decrease in RCT from SR-BI-deficient macrophages (5). However, bone marrow transplantation from SR-BI-deficient mice into LDLR-deficient (19) or apoE-deficient (20) mice resulted in increased atherosclerosis, consistent with an atheroprotective role of hematopoietic SR-BI (which could extend beyond promotion of macrophage cholesterol efflux).

ESTERIFICATION AND DELIVERY OF PERIPHERAL CHOLESTEROL TO THE LIVER FOR EXCRETION IN BILE AND FECES INFLUENCES THE RATE OF MACROPHAGE RCT

Lecithin:cholesterol acyltransferase (LCAT) converts newly effluxed free cholesterol associated with HDL into cholesteryl ester. LCAT has long been believed to be critical for promoting RCT by maintaining a free cholesterol gradient between cells in the periphery and plasma HDL (1). Overexpression of LCAT in mice raises HDL-C levels but resulted in either unaffected (21) or accelerated (22) atherosclerosis. Interestingly, overexpression of LCAT modestly reduced macrophage RCT *in vivo* despite the marked increase in HDL-C levels, at least in part due to a reduction in lipid-poor apoA-I and thus cholesterol efflux via ABCA1 (23). Coexpression of the cholesteryl ester transfer protein

(CETP) with LCAT overexpression reduced atherosclerosis (24), consistent with results of overexpression of LCAT in rabbits, which express CETP (25). Conversely, LCAT knockout mice, despite extremely low levels of HDL-C, have been reported to have reduced or increased atherosclerosis (26, 27). Unexpectedly, LCAT knockout mice have nearly fully preserved macrophage RCT *in vivo*, due at least in part to a high level of lipid-poor apoA-I and robust promotion of efflux via the ABCA1 pathway (23). Studies in healthy normolipidemic humans *in vivo* show that unesterified cholesterol in HDL can be rapidly and directly taken up by the liver and targeted to bile (28), consistent with the ability to maintain RCT independent of cholesteryl ester formation. Thus, while LCAT is clearly important for normal HDL metabolism, it may not be nearly as critical a player in maintaining a normal rate of macrophage RCT as has been traditionally believed.

The pathways by which HDL-C is delivered to the liver also influence the overall rate of RCT. The most direct route involves the selective uptake of HDL-C (both esterified and unesterified) mediated by SR-BI in the liver (Fig. 1), with release of smaller cholesterol-depleted HDL particles that are "recycled" and could potentially be better acceptors of cholesterol efflux from the periphery. Hepatic overexpression of SR-BI markedly reduces plasma HDL-C levels but, in a seeming paradox, substantially reduces atherosclerosis despite the very low levels of HDL-C (29, 30). Conversely, SR-BI knockout mice have markedly increased plasma HDL-C levels due to impaired hepatic catabolism of HDL cholesteryl ester, but have markedly increased atherosclerosis (19). The likely explanation for these observations was provided by the experimental demonstration that overexpression of hepatic SR-BI promoted macrophage RCT, whereas ablation of SR-BI reduced macrophage RCT (31). This is probably the best example of the concept that "flux" of cholesterol through the RCT pathway is a more important determinant of atherosclerosis than steady-state HDL-C concentrations. However, there remain questions regarding the physiologic importance of the hepatic SR-BI pathway for uptake of HDL cholesteryl ester in humans (see below).

Some species, including humans, have an alternative pathway of delivery of HDL-C to the liver, namely that provided by CETP (Fig. 1), which is lacking in rodents. People with loss-of-function mutations in both CETP alleles have extremely high levels of HDL-C, indicating the importance of this pathway in humans. Remarkably, a study in healthy human subjects involving injection of HDL labeled with a CE tracer suggested that the HDL-derived labeled cholesterol that was ultimately excreted into bile was almost all first transferred to apoB-containing lipoproteins (presumably by CETP) (28). Because CETP is a therapeutic target for inhibition to raise plasma levels of HDL-C, the role that CETP plays in modulating the rate of RCT is of considerable interest. Mice engineered to express CETP experience substantial reduction in HDL-C levels. Expression of CETP in mice significantly increased the rate of macrophage RCT (32), consistent with the concept that CETP promotes delivery of HDL-C to the liver. Furthermore, in

SR-BI knockout mice, CETP expression restored the rate of macrophage RCT to normal by providing a “bypass” route for HDL-cholesteryl ester to get to the liver. However, in the absence of LDL receptors, CETP expression actually slowed macrophage RCT by diverting HDL-CE into slowly turning over LDLs. Interestingly, the effect of CETP expression on atherosclerosis in mice is highly variable and dependent on the model used; in general, it is antiatherogenic in models that have relatively intact clearance of apoB-lipoproteins and proatherogenic in models that have markedly impaired apoB-lipoprotein turnover, consistent with the RCT data.

Given that the CETP pathway is a therapeutic target in humans, there is substantial interest in its role in RCT (33). The CETP inhibitor torcetrapib, which raises HDL-C levels, resulted in slower catabolism of HDL apoA-I and did not increase fecal neutral sterol excretion in humans (34). While it has been suggested that HDL from CETP-deficient subjects is dysfunctional in its ability to promote macrophage cholesterol efflux, more recent studies indicate that HDL isolated from CETP-deficient subjects (35) or subjects treated with a CETP inhibitor (36) is more effective than control HDL in promoting macrophage cholesterol efflux via the ABCG1 pathway. Studies of CETP inhibition in rabbits have consistently shown a reduction in atherosclerosis (37). However, a large clinical outcome trial of the CETP inhibitor torcetrapib was terminated early due to significantly increased cardiovascular events and total mortality (38) despite effective raising of HDL-C levels. Complicating the interpretation of these studies, however, is the fact that torcetrapib can raise blood pressure through direct effects on adrenal steroidogenesis (33). The effect of CETP inhibition on RCT and atherosclerosis in humans remains an unresolved, and critically important, question for the field.

The intestine plays a key role in RCT through the ultimate excretion of peripherally derived cholesterol. Indeed, inhibition of intestinal cholesterol absorption with ezetimibe promotes macrophage RCT (39). Some HDL-C may be di-

rectly transferred to the intestine and excreted without first passing through the liver (40), which would represent a change in the classic RCT paradigm.

THE RATE OF MACROPHAGE RCT IN MICE PREDICTS ATHEROSCLEROSIS BETTER THAN THE LEVEL OF HDL-C

The rate of RCT has been challenging to measure in animal models, and most approaches have focused on total peripheral RCT. In order to specifically assay macrophage-specific RCT, the critical atheroprotective pathway, we developed a technique in mice in which macrophages are loaded with cholesterol and labeled with a ³H-cholesterol tracer *ex vivo* and injected intraperitoneally, where they efflux ³H-cholesterol to HDL-based acceptor particles. The appearance of tracer can be followed in plasma and the amount of macrophage-derived tracer excreted in feces can be quantitated as a measure of macrophage-to-feces RCT. Using this approach, apoA-I overexpression was shown to promote (41) and apoA-I deficiency to impair (42) macrophage RCT, consistent with the atheroprotective role of apoA-I. Multiple studies have assessed macrophage-specific RCT in the setting of genetic and pharmacologic manipulation of mice, and the effects on RCT (in contrast to the effects on HDL-C) are largely consistent with the effects of the same interventions on atherosclerosis (**Table 1**). This approach can also be applied to the use of primary mouse (peritoneal or bone marrow-derived) macrophages, allowing the investigation of the role of macrophage-specific genes in RCT *in vivo* as noted above with regard to macrophage ABCA1, ABCG1, and SR-BI (5). Again, the effects on RCT largely mirror the effects on atherosclerosis (Table 1). Overall, the dynamic rate of macrophage RCT correlates much better than steady-state plasma HDL-C level with atherosclerosis, suggesting that it is measuring an atheroprotective

TABLE 1. Relationship between HDL-C, macrophage RCT, and atherosclerosis in response to genetic manipulation and pharmacologic intervention in mice

	Tissue	Effect on HDL-C	Effect on macrophage RCT	Effect on atherosclerosis
Genetic manipulation				
ApoA-I overexpression	Liver	Increase	Increase	Decrease
ApoA-I knockout	Whole body	Decrease	Decrease	Increase
SR-BI overexpression	Liver	Decrease	Increase	Decrease
SR-BI knockout	Whole body	Increase	Decrease	Increase
CETP expression	Liver	Decrease		
LDLR ^{+/+} mice			Increase	Decrease
LDLR ^{-/-} mice			Neutral to decrease	Increase
LCAT overexpression	Liver	Increase	Decrease	Increase
LCAT + CETP expression	Liver	Variable	Neutral	Neutral
LCAT knockout	Whole body	Decrease	Neutral	Variable
ABCA1 knockout	Macrophage	No change	Decrease	Increase
ABCG1 knockout	Macrophage	No change	Decrease	Decrease
ABCA1 + ABCG1 knockout	Macrophage	No change	Decrease	Increase
SR-BI knockout	Macrophage	No change	Neutral	Variable
Pharmacologic intervention				
LXR agonist		Variable	Increase	Decrease
PPAR α agonist		Variable	Increase	Decrease
Ezetimibe		No change	Increase	Decrease

process and could be used to predict the antiatherosclerotic effects of a novel HDL-targeted intervention.

THE IMPORTANCE AND CHALLENGE OF MEASURING RCT IN HUMANS

Robust and sensitive methods for assessing integrated RCT in humans are needed in order to advance insights gained in animals into the human realm and to assess novel therapies targeted toward HDL and RCT. One potential approach is simply to measure the mass of fecal sterol excretion as a surrogate for RCT. For example, an acute intravenous bolus infusion of pro-apoA-I in humans was found to result in a significant increase in fecal sterol excretion, suggesting promotion of RCT (43). However, this approach is not macrophage specific, is unlikely to be very sensitive, and may have limited utility in the chronic steady-state setting due to counter-regulatory pathways involved in biliary cholesterol excretion and fecal sterol absorption.

A steady-state isotope dilution technique has been developed that involves the intravenous infusion of stable-isotopically labeled cholesterol, which is diluted by the efflux of endogenous (unlabeled) cholesterol from tissues into plasma. No primary data are yet available in the peer-reviewed literature, but a more detailed discussion of this method can be found in a recent review (44). It remains to be established whether this method will have utility in assessing RCT in humans following therapeutic intervention. However, its ability to differentiate between hepatic and peripheral (nonhepatic) tissues as the source of cholesterol efflux has not been definitively proven. Furthermore, as with fecal sterol excretion, this method is not macrophage specific. Nevertheless, it represents an interesting new approach to this problem.

An alternative approach would be to load peripheral tissues with a cholesterol-like molecule that is not endogenously synthesized but is effluxed and transported similar to cholesterol and then to follow the rate of disappearance of this tracer from tissues that can be sampled repeatedly (circulating cells, skin, adipose) as well as the rate of excretion in the feces. As with the above methods, however, this is not macrophage specific. Ideally, one would like to label cholesterol specifically in arterial wall macrophages in humans and trace its efflux to plasma through to fecal excretion, a goal that is probably unattainable. However, there may be approaches to selectively labeling cholesterol in macrophages using an in vivo targeting approach that could be adapted to RCT studies in humans. Development of a robust assay for RCT, ideally from the macrophage, in humans will be critical to the development of novel therapeutics that may increase RCT as an antiatherogenic strategy.

SUMMARY

RCT is an essential physiologic process that maintains peripheral and total body cholesterol homeostasis. The relationship between RCT and atherosclerosis has long been

hypothesized, and recent data support the concept that macrophage RCT is mechanistically related to atherogenesis. The molecular mechanisms regulating macrophage RCT have yet to be fully elucidated, and methods for measuring integrated macrophage RCT in humans have yet to be developed and validated. Macrophage RCT remains an attractive target for the development of novel therapies intended to inhibit and regress atherosclerosis. **■**

REFERENCES

1. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9**: 155–167.
2. Rader, D. J. 2006. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J. Clin. Invest.* **116**: 3090–3100.
3. Van Eck, M., I. S. T. Bos, W. E. Kaminski, E. Orso, G. Rothe, J. Twisk, A. Bottcher, E. S. Van Amersfoort, T. A. Christiansen-Weber, W-P. Fung-Leung, et al. 2002. Leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. *Proc. Natl. Acad. Sci. USA.* **99**: 6298–6303.
4. Van Eck, M., R. R. Singaraja, D. Ye, R. B. Hildebrand, E. R. James, M. R. Hayden, and T. J. C. Van Berkel. 2006. Macrophage ATP-binding cassette transporter A1 overexpression inhibits atherosclerotic lesion progression in low-density lipoprotein receptor knockout mice. *Arterioscler. Thromb. Vasc. Biol.* **26**: 929–934.
5. Wang, X., H. L. Collins, M. Ranalletta, I. V. Fuki, J. T. Billheimer, G. H. Rothblat, A. R. Tall, and D. J. Rader. 2007. Macrophage ABCA1 and ABCG1, but not SR-BI, promote macrophage reverse cholesterol transport in vivo. *J. Clin. Invest.* **117**: 2216–2224.
6. Wang, N., D. Lan, W. Chen, F. Matsuura, and A. R. Tall. 2004. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **101**: 9774–9779.
7. Kennedy, M. A., G. C. Barrera, K. Nakamura, A. Baldan, P. Tarr, M. C. Fishbein, J. Frank, O. L. Francone, and P. A. Edwards. 2005. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab.* **1**: 121–131.
8. Baldan, A., L. Pei, R. Lee, P. Tarr, R. K. Tangirala, M. M. Weinstein, J. Frank, A. C. Li, P. Tontonoz, and P. A. Edwards. 2006. Impaired development of atherosclerosis in hyperlipidemic Ldlr^{-/-} and ApoE^{-/-} mice transplanted with Abcg1^{-/-} bone marrow. *Arterioscler. Thromb. Vasc. Biol.* **26**: 2301–2307.
9. Ranalletta, M., N. Wang, S. Han, L. Yvan-Charvet, C. Welch, and A. R. Tall. 2006. Decreased atherosclerosis in low-density lipoprotein receptor knockout mice transplanted with Abcg1^{-/-} bone marrow. *Arterioscler. Thromb. Vasc. Biol.* **26**: 2308–2315.
10. Gelissen, I. C., M. Harris, K. A. Rye, C. Quinn, A. J. Brown, M. Kockx, S. Cartland, M. Packianathan, L. Kritharides, and W. Jessup. 2006. ABCA1 and ABCG1 synergize to mediate cholesterol export to apoA-I. *Arterioscler. Thromb. Vasc. Biol.* **26**: 534–540.
11. Yvan-Charvet, L., M. Ranalletta, N. Wang, S. Han, N. Terasaka, R. Li, C. Welch, and A. R. Tall. 2007. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. *J. Clin. Invest.* **117**: 3900–3908.
12. Naik, S. U., X. Wang, J. S. Da Silva, M. Jaye, C. H. Macphee, M. P. Reilly, J. T. Billheimer, G. H. Rothblat, and D. J. Rader. 2006. Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo. *Circulation.* **113**: 90–97.
13. Joseph, S. B., E. McKilligin, L. Pei, M. A. Watson, A. R. Collins, B. A. Laffitte, M. Chen, G. Noh, J. Goodman, G. N. Hagger, et al. 2002. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl. Acad. Sci. USA.* **99**: 7604–7609.
14. Levin, N., E. D. Bischoff, C. L. Daige, D. Thomas, C. T. Vu, R. A. Heyman, R. K. Tangirala, and I. G. Schulman. 2005. Macrophage liver X receptor is required for antiatherogenic activity of LXR agonists. *Arterioscler. Thromb. Vasc. Biol.* **25**: 135–142.
15. Chawla, A., W. A. Boisvert, C. H. Lee, B. A. Laffitte, Y. Barak, S. B. Joseph, D. Liao, L. Nagy, P. A. Edwards, L. K. Curtiss, et al. 2001. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol. Cell.* **7**: 161–171.
16. Chinetti, G., S. Lestavel, V. Bocher, A. T. Remaley, B. Neve, I. P. Torra, E. Teissier, A. Minnich, M. Jaye, N. Duverger, et al. 2001. PPAR-

- alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat. Med.* **7**: 53–58.
17. Li, A. C., C. J. Binder, A. Gutierrez, K. K. Brown, C. R. Plotkin, J. W. Pattison, A. F. Valledor, R. A. Davis, T. M. Willson, J. L. Witztum, et al. 2004. Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARalpha, beta/delta, and gamma. *J. Clin. Invest.* **114**: 1564–1576.
18. Rothblat, G. H., M. de la Llera-Moya, V. Atger, G. Kellner-Weibel, D. L. Williams, and M. C. Phillips. 1999. Cell cholesterol efflux: integration of old and new observations provides new insights. *J. Lipid Res.* **40**: 781–796.
19. Covey, S. D., M. Krieger, W. Wang, M. Penman, and B. L. Trigatti. 2003. Scavenger receptor class B type I-mediated protection against atherosclerosis in LDL receptor-negative mice involves its expression in bone marrow-derived cells. *Arterioscler. Thromb. Vasc. Biol.* **23**: 1589–1594.
20. Zhang, W., P. G. Yancey, Y. R. Su, V. R. Babaev, Y. Zhang, S. Fazio, and M. F. Linton. 2003. Inactivation of macrophage scavenger receptor class B type I promotes atherosclerotic lesion development in apolipoprotein E-deficient mice. *Circulation.* **108**: 2258–2263.
21. Mehlum, A., E. Gjernes, L. A. Solberg, T. A. Hagve, and H. Prydz. 2000. Overexpression of human lecithin:cholesterol acyltransferase in mice offers no protection against diet-induced atherosclerosis. *APMIS.* **108**: 336–342.
22. Berard, A. M., B. Foger, A. Remaley, B. L. Vaisman, G. Talley, B. Paigen, R. F. J. Hoyt, H. B. J. Brewer, and S. Santamarina-Fojo. 1997. High plasma HDL concentration associated with enhanced atherosclerosis in transgenic mice overexpressing lecithin-cholesterol acyltransferase. *Nat. Med.* **3**: 744–749.
23. Tanigawa, H., J. T. Billheimer, J. Tohyama, I. Fuki, D. Ng, G. Rothblat, and D. J. Rader. 2009. LCAT expression has minimal effects on macrophage reverse cholesterol transport in vivo. *Circulation.* In press.
24. Foger, B., M. Chase, M. J. Amar, B. L. Vaisman, R. D. Shamburek, B. Paigen, J. Fruchart-Najib, J. A. Paiz, C. A. Koch, R. F. Hoyt, et al. 1999. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. *J. Biol. Chem.* **274**: 36912–36920.
25. Hoeg, J. M., S. Santamarina-Fojo, A. Berard, J. F. Cornhill, E. Herderick, S. Feldman, C. Haudenschild, B. Vaisman, R. F. J. Hoyt, S. Demosky, et al. 1996. Overexpression of lecithin:cholesterol acyltransferase in transgenic rabbits prevents diet-induced atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **93**: 11448–11453.
26. Lambert, G., N. Sakai, B. L. Vaisman, E. B. Neufeld, B. Marteyn, C. C. Chan, B. Paigen, E. Lupia, A. Thomas, L. J. Striker, et al. 2001. Analysis of glomerulosclerosis and atherosclerosis in lecithin cholesterol acyltransferase-deficient mice. *J. Biol. Chem.* **276**: 15090–15098.
27. Furbee, J. W., Jr., J. K. Sawyer, and J. S. Parks. 2002. Lecithin:cholesterol acyltransferase deficiency increases atherosclerosis in the low density lipoprotein receptor and apolipoprotein E knockout mice. *J. Biol. Chem.* **277**: 3511–3519.
28. Schwartz, C. C., J. M. VandenBroek, and P. S. Cooper. 2004. Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans. *J. Lipid Res.* **45**: 1594–1607.
29. Kozarsky, K. F., M. H. Donahee, J. M. Glick, M. Krieger, and D. J. Rader. 2000. Gene transfer and hepatic overexpression of the HDL receptor SR-BI reduces atherosclerosis in the cholesterol-fed LDL receptor-deficient mouse. *Arterioscler. Thromb. Vasc. Biol.* **20**: 721–727.
30. Arai, T., N. Wang, M. Bezouevski, C. Welch, and A. R. Tall. 1999. Decreased atherosclerosis in heterozygous low density lipoprotein receptor-deficient mice expressing the scavenger receptor BI transgene. *J. Biol. Chem.* **274**: 2366–2371.
31. Zhang, Y., J. R. Da Silva, M. Reilly, J. T. Billheimer, G. H. Rothblat, and D. J. Rader. 2005. Hepatic expression of scavenger receptor class B type I (SR-BI) is a positive regulator of macrophage reverse cholesterol transport in vivo. *J. Clin. Invest.* **115**: 2870–2874.
32. Tanigawa, H., J. T. Billheimer, J. Tohyama, Y. Zhang, G. Rothblat, and D. J. Rader. 2007. Expression of cholesteryl ester transfer protein in mice promotes macrophage reverse cholesterol transport. *Circulation.* **116**: 1267–1273.
33. Rader, D. J. 2007. Illuminating HDL—is it still a viable therapeutic target? *N. Engl. J. Med.* **357**: 2180–2183.
34. Brousseau, M. E., M. R. Diffenderfer, J. S. Millar, C. Nartsupha, B. F. Asztalos, F. K. Welty, M. L. Wolfe, M. Rudling, I. Bjorkhem, B. Angelin, et al. 2005. Effects of cholesteryl ester transfer protein inhibition on high-density lipoprotein subspecies, apolipoprotein A-I metabolism, and fecal sterol excretion. *Arterioscler. Thromb. Vasc. Biol.* **25**: 1057–1064.
35. Matsuura, F., N. Wang, W. Chen, X. C. Jiang, and A. R. Tall. 2006. HDL from CETP-deficient subjects shows enhanced ability to promote cholesterol efflux from macrophages in an apoE- and ABCG1-dependent pathway. *J. Clin. Invest.* **116**: 1435–1442.
36. Van-Charvet, L., F. Matsuura, N. Wang, M. J. Bamberger, T. Nguyen, F. Rinninger, X. C. Jiang, C. L. Shear, and A. R. Tall. 2007. Inhibition of cholesteryl ester transfer protein by torcetrapib modestly increases macrophage cholesterol efflux to HDL. *Arterioscler. Thromb. Vasc. Biol.* **27**: 1132–1138.
37. Rader, D. J. 2004. Inhibition of cholesteryl ester transfer protein activity: a new therapeutic approach to raising high-density lipoprotein. *Curr. Atheroscler. Rep.* **6**: 398–405.
38. Barter, P. J., M. Caulfield, M. Eriksson, S. M. Grundy, J. J. Kastelein, M. Komajda, J. Lopez-Sendon, L. Mosca, J. C. Tardif, D. D. Waters, et al. 2007. Effects of torcetrapib in patients at high risk for coronary events. *N. Engl. J. Med.* **357**: 2109–2122.
39. Sehayek, E., and S. L. Hazen. 2008. Cholesterol absorption from the intestine is a major determinant of reverse cholesterol transport from peripheral tissue macrophages. *Arterioscler. Thromb. Vasc. Biol.* **28**: 1296–1297.
40. Groen, A. K., R. P. Oude Elferink, H. J. Verkade, and F. Kuipers. 2004. The ins and outs of reverse cholesterol transport. *Ann. Med.* **36**: 135–145.
41. Zhang, Y., I. Zanotti, M. P. Reilly, J. M. Glick, G. H. Rothblat, and D. J. Rader. 2003. Overexpression of apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces in vivo. *Circulation.* **108**: 661–663.
42. Moore, R. E., M. Navab, J. S. Millar, F. Zimetti, S. Hama, G. H. Rothblat, and D. J. Rader. 2005. Increased atherosclerosis in mice lacking apolipoprotein A-I attributable to both impaired reverse cholesterol transport and increased inflammation. *Circ. Res.* **97**: 763–771.
43. Eriksson, M., L. A. Carlson, T. A. Miettinen, and B. Angelin. 1999. Stimulation of fecal steroid excretion after infusion of recombinant proapolipoprotein A-I; potential reverse cholesterol transport in humans. *Circulation.* **100**: 594–598.
44. deGoma, E. M., R. L. deGoma, and D. J. Rader. 2008. Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *J. Am. Coll. Cardiol.* **51**: 2199–2211.