Lymphatics as a New Active Player in Reverse Cholesterol Transport

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Reverse cholesterol transport (RCT), a key function of high-density lipoproteins (HDL), prevents excess cholesterol in tissues. A study in this issue (Lim et al., 2013) suggests that lymphatic vessels are critical for normal RCT and mediate the active *trans*-endothelial transport of HDL via the HDL receptor SR-BI.

Cells require cholesterol for viability. However, excess cholesterol can be toxic to cells and therefore cells have evolved mechanisms to promote cholesterol efflux in a regulated manner. Cellular cholesterol is effluxed to "acceptors" that are generally in the family of high-density lipoproteins (HDL), including its major protein apolipoprotein A-I (apoA-I). Once cellular cholesterol is effluxed to HDL, it is returned to liver for excretion in the bile in a process known as "reverse cholesterol transport (RCT)" (Rader et al., 2009). RCT is not only a physiological process required for maintaining cholesterol homeostasis but also has potential protective effects on atherosclerosis. While substantial attention has been focused on the initial pathways of cellular cholesterol efflux and on the fate of HDL cholesterol within the blood, much less is known about the fate of the newly effluxed cholesterol and how it reaches the blood. Lim et al. (2013) report that impaired lymphatic function reduces the efficiency of RCT and that lymphatic endothelium actively transports HDL particles.

Lymphatic vessels transport interstitial fluid and cells back to the blood, coordinating adaptive immune responses, and carry lipids absorbed from the intestine to the blood. Interstitial and lymphatic fluid contains HDL and apoA-I (Sloop et al., 1987), and lymphatics have long been suspected to play an important role in transporting newly effluxed cholesterol from the site of efflux to the blood (Sloop et al., 1987). However, since molecular tools to investigate lymphatic vessel function were only recently developed, the physiological roles for this vascular network remain to be rigorously defined. Effects of lymphatic loss of function on lipid metabolism, such as obesity and the deposition of fat in edematous tissue, have been observed in mice lacking lymphatic function due to genetic (e.g., loss of the lymphatic endothelial transcription factor PROX1 [Harvey et al., 2005]) or mechanical reasons (Rutkowski et al., 2006), and in humans with chronic lymphedema (Schirger et al., 1962). Lim et al. (2009) reported in an earlier study that hypercholesterolemic apoE-deficient mice have abnormal lymphatic function that results in excess accumulation of cholesterol in the periphery. These studies hinted at important connections between obesity, cholesterol, and lymphatic function, but clear molecular links were not established.

Lim et al. (2013) now establish a convincing causal connection between lymphatic vessel function and HDL transport and provide a molecular basis for this observation. By adapting an established method for assessing macrophage RCT in vivo (Rader et al., 2009), they show that after injection of macrophages labeled with fluorescent cholesterol into the footpad, apoE-deficient mice have markedly impaired cholesterol transport into plasma and liver. They further demonstrate that treatment of apoE-deficient mice with ezetimibe, which reduces plasma cholesterol, or local injection of VEGF-C to promote lymphatic formation, improves lymphatic function, reduces peripheral cholesterol accumulation, and, most compellingly, promotes RCT. In order to test whether this process is dependent on HDL-mediated transport, the authors show that labeled HDL injected into the footpad is rapidly taken up by lymphatic vessels and eventually transported to the blood—and that surgical disruption of lymphatic drainage markedly impairs entry of injected HDL into the blood. Furthermore, apoE-deficient mice exhibit a defect in transport of HDL into lymphatics that is improved by both ezetimibe and VEGF-C treatment. These studies clearly implicate lymphatic vessels in mediating the return of macrophage-derived cholesterol and interstitial fluid HDL to the circulation, reliably indicating an important role in RCT.

The authors then investigate whether lymphatic vessels are simply conduits that passively collect the interstitial fluid HDL into lymph, or whether they actively participate in HDL transport. Using lymphatic endothelial cells (LECs), they find evidence for expression of the classic HDL receptor scavenger receptor class BI (SR-BI) (Figure 1). SR-BI not only promotes uptake of HDL cholesterol into hepatocytes but also has HDL transport functions in other cell types, including vascular endothelial cells. Lim et al. (2013) show that LECs internalize and transcytose HDL and that this process is blocked by inhibition of SR-BI with siRNA, an antibody, or a small molecule. Compellingly, after injection of cholesterol-labeled macrophages in the footpad, appearance of labeled cholesterol in lymph, lymph nodes, and plasma is markedly diminished if SR-BI is inhibited by antibody treatment or deleted genetically. These findings suggest that the lymphatic vessels are not mere passive conduits but function as active biological transporters of HDL from interstitial fluid into lymph, thus actively regulating RCT.

Lim et al. (2013) have thus identified a novel role for lymphatic vessels in RCT that may connect lymphatic function to

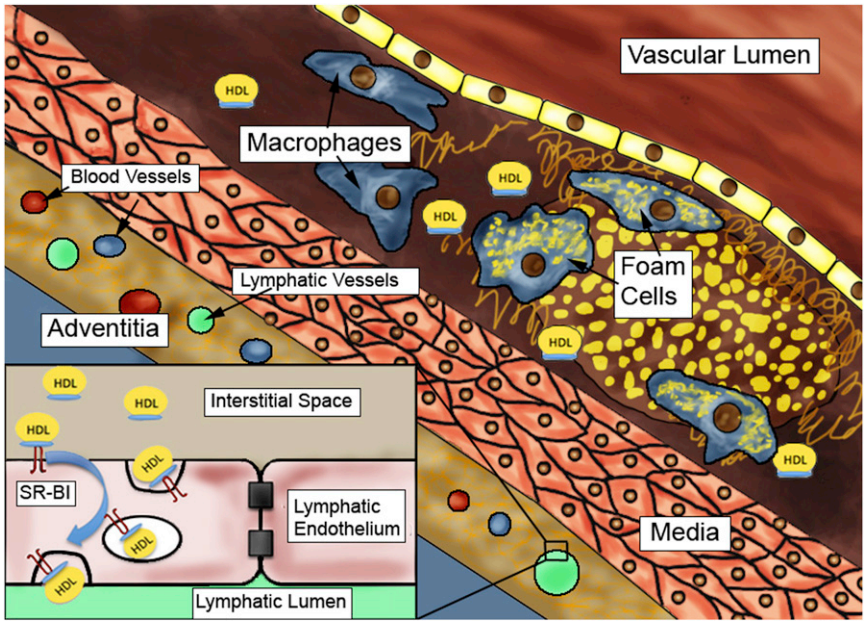

Figure 1. Role of Lymphatics in Uptake and Transport of Interstitial Fluid HDL
HDL promotes efflux of cholesterol from macrophages, here shown conceptually within the atherosclerotic arterial plaque (not directly demonstrated in the current article by Lim et al. [2013]). HDL in interstitial fluid in the adventitia is actively transported by the lymphatic endothelium via SR-BI into the lumen of lymphatic vessels, whereupon it is ultimately returned to the plasma compartment.

atherosclerosis in humans. However, several important questions remain to be fully addressed. For example, how quantitatively important is lymphatic function in reverse cholesterol transport? The authors make extensive use of apoE-deficient animals to address this question. However, the basis for the observed lymphatic defects in apoE-deficient mice is not yet clear, and it would be important to address the role of lymphatics in cholesterol transport using mice lacking lymphatic function through a mechanism independent of cholesterol metabolism, for example with partial loss of VEGFC-VEGFR3 signaling or PROX1. Along the same lines, SR-BI is a strong candidate mechanism for HDL uptake and transport by lymphatic vessels, but additional studies such as selective loss of SR-B1 in lymphatic endothelial cells in vivo are required to confirm that HDL transport by lymphatics reflects direct uptake by lymphatic endothelium rather than passive movement with interstitial fluid or an indirect cellular mechanism. Importantly, what is the relationship of lymphatic function to atherosclerosis (Figure 1)? It was previously shown that macrophages and dendritic cells can egress from the regressing atherosclerotic plaque via the lymphatic system (Llodrá et al., 2004); now, it appears that HDL itself may do the same. Might conditions of impaired lymphatic function exacerbate atherosclerosis—and, conversely, might treatment to improve lymphatic function be a novel therapeutic approach to atherosclerotic disease?

Several recent events have created substantial uncertainty regarding the clinical benefits of raising plasma levels of HDL cholesterol to reduce risk of atherosclerotic vascular disease (Rader and Tall, 2012). These include human genetic studies showing that genes influencing HDL-C levels have a poor relationship to vascular disease, as well as several failed clinical trials of HDL-raising drugs. Nevertheless, there remains substantial interest in the therapeutic targeting of cholesterol efflux and RCT as a strategy to combat atherosclerosis (Degoma and Rader, 2011). The concept that lymphatic vessels play an important biological role in this process opens new vistas for therapeutic intervention. A fact brought home strongly by these exciting new studies is that there remains much that we don't know about the roles of lymphatic vessels in physiologic and pathologic states. Further studies of mouse models and the development of new tools to observe and measure lymphatic function in human patients are certain to reveal additional dynamic roles for lymphatic vessels and perhaps unexpected strategies for the treatment of common human diseases.

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