Editorial Commentary

The "HDL receptor" scavenger receptor class B type 1 finesses the uptake of low-density lipoproteins into the subendothelial space of arteries

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Atherosclerosis is a chronic inflammation initiated by the accumulation of low-density lipoproteins (LDL) in the subendothelial space of the artery wall in spatially selected regions of the artery. The LDL is derived from circulating plasma lipoproteins. The passage of macromolecules across the endothelial barrier may involve one of three mechanisms—diffusional, paracellular or transcellular. An endothelial barrier with tight junctions will not allow transfer by the first two of these mechanisms. The transcellular mechanism or transcytosis (that is the movement through the endothelial cytoplasm from the luminal to the abluminal surface of the cells) involves either indirect transfer through recycling of endosomal vesicles that bypasses the degradative lysosome or direct transcellular movement in vesicles. Clarification of the mechanisms responsible for the transport of LDL from the plasma across the endothelium has not emerged until recently. Three pathways have been implicated in the transcytosis of LDL: caveolin-1, activin-like kinase 1 (ALK1) and scavenger receptor class B type 1 (SR-B1) (1). Recycling endosomes are part of the endocytic cycle of the LDL receptor (LDLR), but since LDL is found in the subendothelial space in the absence of the LDLR in mice, this is not a required pathway for LDL transcytosis. In a recent paper published in *Nature* from Philip Shaul's laboratory the mechanism of LDL transcytosis into the artery wall has been probed in much more detail (2). They identify endothelial *SR-B1* as the receptor responsible for LDL transcytosis and identify cellular proteins that participate in this pathway.

SR-B1 is a widely expressed scavenger receptor. It was initially thought of as the "HDL receptor" because the binding of HDL to the receptor results in selective cholesteryl ester movement into cells. Selective uptake of cholesteryl esters from HDL in the liver is a central element of the process of reverse cholesterol transport (3). While this receptor is highly expressed in the liver and steroidogenic tissues, it is also expressed in many other tissues and cell types, including endothelial cells. SR-B1 gene expression is regulated by a number of transcription factors including SREBP1 and at least five microRNAs (miRNA) that regulate its translation by binding to the 3'-UTR of the mRNA. The protein undergoes substantial post-translational modification resulting in a notable increase in the mature protein size compared to the primary translational product. The mature protein is associated with the plasma membrane as a single large extracellular loop with two transmembrane domains and a short N-terminal cytoplasmic tail and a larger C-terminal cytoplasmic tail (4).

In a cell culture system involving either murine aortic endothelial cells or human coronary artery endothelial cells, it has been shown that the transcytosis of LDL is mediated by SR-B1 (5). In addition, aortas from SR-B1 knockout mice accumulate less subendothelial LDL *ex vivo*. To dissect the elements of SR-BI required for LDL transcytosis Huang *et al*. (2) knocked down endogenous SR-B1 in human arterial endothelial cells. The cells were complemented with either wild type SR-B1 or selected SR-B1 mutants and plated in transwells for the assessment of LDL transcytosis.

These studies revealed that there were residues in the N-terminal portion of the extracellular loop that were necessary for LDL binding. In addition, they identified a highly conserved octapeptide (IQAYSESL) in the proximal C-terminal cytoplasmic tail that was essential for transcytosis. This octapeptide is a binding site for the guanine nucleotide exchange factor dedicator of cytokinesis 4 (DOCK4) which led to the activation of Rac1 (a RHO GTPase). Activation of Rac1 resulted in the internalization of the LDL-SR-B1 complex and its ultimate discharge on the abluminal face of the endothelial cell. Colocalization of LDL, SR-B1 and DOCK4 was demonstrated in the endothelial cells. Thus, DOCK4 serves as an SR-B1 adaptor molecule to mediate LDL transcytosis in endothelial cells.

Previous studies in the liver have shown that SR-B1 is associated with the adaptor protein PDZK1. In the absence of PDZK1 hepatic SR-B1 levels are reduced by 95% (6). Huang *et al*. demonstrated that PDZK1 is not required for LDL transcytosis by endothelial cells (2). A second "receptor" for LDL is ALK1, a member of the TGF type 1 receptor family, that binds endothelial cells, perhaps including those of LDL with low affinity and high capacity (7). The kinase function of the receptor is not required for LDL binding. In the absence of SR-B1 no change in ALK1 expression was observed (2). Although LDL transcytosis is more dramatically reduced in the absence of both SR-B1 and ALK1, the absence of SR-B1 significantly reduces LDL transcytosis but does not alter the expression of ALK1 (2). This indicates that SR-B1 is the primary receptor required for LDL transcytosis in endothelial cells.

HDL transits the endothelial cells, perhaps including those of the lymphatics associated with the artery wall (8), into the intimal space where it may be remodeled to generate cholesterol acceptors for cholesterol efflux and initiation of reverse cholesterol transport. As SR-B1 is known as an HDL receptor, what role does SR-B1 have for the transcytosis of HDL? HDL competes with LDL for binding to SR-B1. HDL transcytosis through arterial endothelial cells is dependent on SR-B1 and ABCG1 (9), but is independent of PDZK1 and DOCK4 (2). At least in brain microvascular endothelial cells it is also independent of caveolae and clathrin (10).

The vital role of SR-B1 in atherosclerosis research was highlighted by the discovery that mice deficient in both *Apoe* and *Scar1* (*Scar1*$^{-/-}$ *Apoe*$^{-/-}$) developed profound obstructive coronary atherosclerosis resulting in myocardial infarction and premature mortality (11). This model is especially notable as most of the genetic manipulations in atherogenic mouse models do not induce coronary artery atherosclerotic lesions. Although this phenotype is not fully understood, it does suggest very strongly that SR-B1 is atheroprotective and that SR-B1 mediated LDL transcytosis is unlikely to be an important mediator of the coronary atherosclerosis in this model, though the role of the LDLR and ALK1 cannot yet be excluded. As mentioned above PDZK1 is not involved in LDL transcytosis, but it has been implicated in atherogenesis via modification of cholesterol homeostasis. Thus, no coronary lesions are seen when *Pdzk1*$^{-/-}$ *Apoe*$^{-/-}$ mice are fed a western type diet although coronary lesions develop when the double knockout mice are fed the Paigen diet containing 1.25% cholesterol and 0.5% Na cholate (6). In sum, it seems that a role for SR-B1 in endothelial cell LDL transcytosis is not critical for the enhanced coronary artery atherosclerosis observed in the *Scar1*$^{-/-}$ *Apoe*$^{-/-}$ mice.

So what is the *in vivo* significance of LDL transcytosis. To address this Huang *et al*. (2) generated an *Apoe* deficient mouse lacking SR-B1 expression selectively in endothelial cells. They found that atherosclerosis was significantly reduced. Furthermore, in acute experiments, fluorescently labeled LDL does not cross into the intima in the endothelial cell specific *Scarb1* knockout mouse. Additionally, when the aortas were isolated from these mice, they were not efficient in the transcytosis of LDL. Thus SR-B1 expression in endothelial cells is pro-atherogenic.

It is well established that atherosclerotic lesions develop in specific sites in the aorta, largely determined by hemodynamic forces (12). For example, the lesser curvature of the aortic arch is atheroprone while the greater curvature is atheroresistant. One of the fascinating aspects of the findings of Huang *et al*. (2) is that transcytosis of LDL is distinct at these two aortic regions as is also the expression of both SR-B1 and DOCK4, with these proteins being more highly expressed in the atheroprone lesser curvature. The precise molecular basis for these findings is not clear. Biomechanics of blood flow is known to affect epigenetic modification of the endothelial cell DNA (13) and this could be a mechanism, though there is no direct basis for implicating this mechanism in relation to the differential expression of these two proteins. None of the known flow sensitive transcription factors are known to influence SR-B1 or DOCK4 expression. Another possible mechanism for this regulation may be through miRNA. However, the hitherto described flow sensitive miRNAs (14) show no

overlap with those thought to regulate SR-B1 expression (3).

A study on caveolin and transcytosis related to the differential phenotype of atheroprone and atheroresistant regions was very recently reported (15). The knockout of caveolin in atherosclerotic mouse models has a complex phenotype including the reduction of atherosclerosis, evident especially with very early lesions. Transcytosis of LDL was notably reduced in *Cav1*$^{-/-}$/*Ldlr*$^{-/-}$ mice, especially in the atheroprone lesser curvature of the arch. Morphological examination of the aortic arch in *Ldlr*$^{-/-}$ mice revealed that in this region the caveolae were reduced on the luminal surface with more of these vesicles being intracellular compared to the atheroresistant greater curvature of the arch without a difference in the total caveolae.

In humans it is known that premenopausal females have less atherosclerosis than males of similar age. LDL transcytosis in human coronary endothelial cells from males is higher than in cells from premenopausal females (16,17). Estrogen treatment of cells from males but not premenopausal females reduces transcytosis and SR-B1 but has no effect on either caveolin-1 or ALK1. Notably, estrogen has no effect on transcytosis in SR-B1 knockdown cells. Estrogen repression of SR-BI is apparently mediated by the G-protein coupled estrogen receptor. Interestingly, the SR-B1 promoter has estrogen response elements. These effects were not seen in murine endothelial cells. Thus, at least in humans, estrogen's effects on LDL transcytosis may contribute to the gender differences in atherosusceptibility.

In summary, the paper by Huang *et al*. (2) highlights a number of important points related to the initiation of atherosclerosis:

(I) SR-B1 and DOCK4 are involved in the transcytosis of LDL across arterial endothelial cells accounting for a pro-atherogenic role of the receptor;

(II) In contrast to the role of SR-B1 in endothelial cells, the receptor expressed in the liver and macrophages is atheroprotective;

(III) The SR-B1 function in the endothelial cells is independent of the adaptor protein PDZK1, while that in the liver is highly dependent;

(IV) The detailed pathways for LDL transcytosis and HDL transcytosis across endothelial cells differ from one another;

(V) Transcytosis of LDL is a process that is distinguished between atheroprone and atheroresistant regions of the aorta, accompanied by differences in the expression levels of SR-B1 and DOCK4.

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Footnote

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