# A new pathway of macrophage cholesterol efflux

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Macrophage foam cells (i.e., cholesteryl ester-laden macrophages) are abundant in atherosclerotic plaques, and increased macrophage foam cell content is associated with plaque instability (1, 2). Macrophages are generally thought to unload surplus cholesterol via efflux mediated by the ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1) to apolipoprotein A1 [apoA1 (3, 4)] and high-density lipoproteins [HDLs (5, 6)], respectively. Several studies in large-population cohorts have shown that the cholesterol efflux capacity of HDL (i.e., its potential to act as an acceptor for cholesterol efflux from macrophages) is an inverse predictor of cardiovascular disease (7–9), highlighting the importance of cholesterol efflux as an atheroprotective mechanism.

In PNAS, He et al. (10) describe a mechanism for macrophage cholesterol efflux, which involves the transfer of surplus cholesterol from the macrophage plasma membrane to the plasma membrane and cytosolic lipid droplets of adjacent smooth muscle cells (SMCs). This transcellular cholesterol movement (TCM) could represent a mode that macrophages and perhaps other cell types use to unload excessive cholesterol, helping to prevent potential toxicity associated with excessive cholesterol accumulation and in atherosclerotic plaques helping to reverse the formation of macrophage foam cells. The discovery of TCM was made possible by the use of sophisticated technology (nano-secondary ion mass spectrometry [NanoSIMS] imaging) that allows microscopic colocalization of $^{13}$C-cholesterol with $^{15}$N-choline (11). As such, the transfer of $^{13}$C-cholesterol from macrophages to adjacent SMCs that were metabolically labeled with $^{15}$N-choline could be imaged ($^{15}$N-choline is incorporated into the SMC membrane components phosphatidylcholine and sphingolipids). Within atherosclerotic plaques, macrophages or monocytes could unload their surplus cholesterol onto adjacent SMCs, leading to reversal of macrophage foam cell formation, which may decrease atherosclerotic plaque growth and instability. At a mechanistic level, cholesterol transfer from macrophages to SMCs still occurred after initial depletion of macrophage membrane cholesterol by methyl-β-cyclodextrin (10), indicating that it was not simply the consequence of nonphysiologic macrophage plasma membrane cholesterol overload. The transfer was abolished when cells were cultured at 4 °C instead of 37 °C (10), suggesting the necessity of a metabolically active process. This could involve secretion of cholesterol vesicles onto extracellular

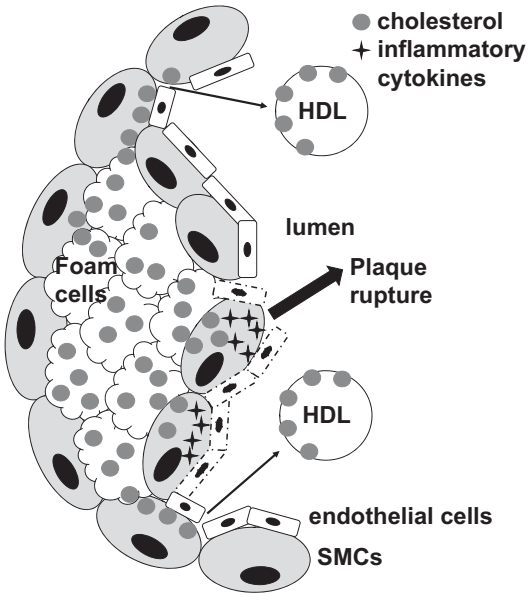


**Fig. 1. Transcellular cholesterol movement (TCM) from macrophage foam cells to smooth muscle cells (SMCs) in the atherosclerotic plaque and potential consequences for plaque stability. TCM from foam cells to medial SMCs expressing high levels of ATP-binding cassette transporters A1 (ABCA1) leads to cholesterol efflux to high-density lipoprotein (HDL; *Top* and *Bottom*). TCM from foam cells to intimal SMCs expressing low levels of ABCA1 may lead to SMC foam cell formation and cytokine secretion, potentially causing plaque rupture (*Middle*).**

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matrix, such as collagen IV or dead cells, as shown by the authors in previous work (12). He et al. (10), however, state this may be challenging to prove and propose direct cell–cell interaction via tunneling nanotubes. Indeed, these tunneling nanotubes exist in myeloid cells and originate from threads of membranes (13). Although highly speculative, tunneling nanotubes may connect macrophages and SMCs and allow for cholesterol particle shuttling. This transfer may, however, be bidirectional (13), and therefore it will be important to investigate whether cocultures of cholesterol-laden macrophages with SMCs also lead to increased cholesterol mass in SMCs or to formation of larger or more numerous lipid droplets.

The cholesterol transfer from macrophages to SMCs occurred in the absence of serum or HDL (10). It would be of interest to investigate whether this transfer still occurs when serum or HDL are added, because this may resemble the in vivo setting more closely. However, this may require the identification of proteins that have an essential role in TCM. In this regard, macrophage *Abca1* deficiency did not prevent TCM from macrophages to SMCs (10), suggesting the mechanism was independent of known cholesterol efflux pathways. Nonetheless, *Abca1*$^{-/-}$ macrophages show increased *Abcg1* expression (14). It may be worthwhile to assess cholesterol transfer from macrophages with combined *Abca1* and *Abcg1* deficiency to rule out the contribution of known cholesterol efflux pathways. Indeed, some lines of evidence indicate that ABCA1/G1 could contribute to particulate cholesterol efflux. The authors have shown previously that liver X receptor (LXR) agonists increase the cholesterol content of particles secreted by macrophages onto extracellular matrix (11), consistent with a role for the LXR target genes *Abca1* and *Abcg1* (5, 15). The same study showed that HDL reduced the cholesterol content of the particles on the extracellular matrix (11). Although macrophage *Abca1* and *Abcg1* mediate the majority of cholesterol efflux to HDL [~60 to 70% (1, 14)], additional pathways may be involved, as proposed by the authors (10, 11). This could be a likely scenario in the setting of *Abca1* and *Abcg1* deficiency where cholesterol mostly accumulates in the plasma membrane (1, 16). Other cell surface proteins could be involved in cholesterol efflux to HDL and transfer to SMCs, as suggested by the authors (10), and if elucidated, genetic interventions may allow for investigation of the significance of TCM in vivo.

A major question is to what extent TCM plays a role in atherosclerotic plaques in vivo. The presence of extracellular cholesterol microdomains, as identified by Kruth et al. (17), has been demonstrated in the vicinity of foam cells in human aortic tissue using immunostaining (18). However, these microdomains consist of branching irregularly shaped deposits and, although also containing extracellular cholesterol, were thought to be different from the spherical cholesterol particles identified by He et al. (11). It has been shown that cholesterol-rich diet feeding increases the width of the membrane bilayer of SMCs that correlates with an increased cholesterol/phospholipid ratio in atherosclerotic plaques in rabbits (19). The study by He et al. (10) would imply that SMC membrane cholesterol enrichment in atherosclerotic plaques could be the consequence of TCM from plaque macrophages.

**In PNAS, He et al. describe a mechanism for macrophage cholesterol efflux, which involves the transfer of surplus cholesterol from the macrophage plasma membrane to the plasma membrane and cytosolic lipid droplets of adjacent smooth muscle cells (SMCs).**

Whether this transfer is beneficial in atherogenesis may depend on the specific location of the macrophages and SMCs in the plaque (Fig. 1). Studies by the Francis laboratory (20) have shown that medial SMCs express high levels of ABCA1 whereas ABCA1 is low in intimal SMCs of human plaques. These data suggest that unloading of macrophage cholesterol to medial SMCs may represent an antiatherogenic mechanism, because cholesterol could be readily effluxed to HDL via ABCA1. However, transfer of cholesterol to intimal SMCs could have the opposite effect. Because ABCA1 expression is low, the transfer likely enhances SMC cholesterol accumulation, potentially leading to the conversion of intimal SMCs into macrophage-like cells (21, 22). These cells may start to secrete proinflammatory cytokines, rather than promoting plaque stability via production of collagen (21, 22), and as such could induce formation of unstable atherosclerotic plaques (Fig. 1). Recent studies have also suggested that macrophage foam cell formation may not always be proatherogenic. In a study by Kim et al. (23), suppression of inflammatory gene expression was observed in aortic macrophage foam cells, with increased inflammatory gene expression in non-foam cell macrophages. This could be attributable to desmosterol accumulation in macrophage foam cells, which has previously been shown by the Glass laboratory (24) to suppress macrophage inflammation via activation of LXR. Nonetheless, the studies by He et al. mainly focus on membrane cholesterol efflux, which, as shown by studies in macrophages with *Abca1* and *Abcg1* deficiency, has a highly antiinflammatory and antiatherogenic role (1, 2).

In sum, these studies provide an important mechanism for macrophage cholesterol efflux involving TCM as shown using the elegant NanoSIMS imaging technique. The identification of pathways and cell surface proteins regulating this process may allow future research on the significance of TCM in atherosclerotic plaque formation in vivo.


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