Atherosclerosis is a complex disease that involves many cell types and biochemical pathways. In the research literature, however, there is a continued emphasis on the importance of lipoprotein interactions with vascular wall cells as a prominent mechanism that underlies all stages of the disease. Initial studies on cellular lipoprotein interactions described a limited number of receptors, primarily the LDL and scavenger receptors. These receptors were believed to act only in a transport function, to facilitate the transfer of particles to the lysosome. Within the lysosome, lipoprotein particles were degraded and cholesterol was transferred to the cytosol, where it was re-esterified. This stored cholesterol ester was subsequently removed by many extracellular acceptor molecules. However, each stage of this process is more complex than was originally envisaged. One aspect of this complexity is the increasing number of proteins that have been described as receptors for native or modified lipoproteins. In addition, papers that are featured in the present commentary provided other new features of cellular lipoprotein metabolism. These include the ability of lipoprotein receptors to be influenced by signaling pathways, the complex response of the cell to intracellular cholesterol storage, and the importance of the properties of the extracellular acceptor molecules to the removal of cholesterol from the cell.

Lipoproteins both initiate and are modulated by signaling pathways, although the details of these regulatory mechanisms are relatively scant [1,2]. The effect of signaling on lipoprotein receptor activity in macrophages has the potential to be particularly profound, given the large number of cytokines that may interact with this cell type in atherosclerotic lesions [3]. Macrophage colony-stimulating factor (M-CSF) is one of the cytokines that are prominent in macrophage-rich lesions [4]. Whitman et al. [5*] observed that acute exposure of cultured macrophages to M-CSF augmented cholesterol ester synthesis during incubation with β-VLDL. The rapidity of this augmentation inferred a post-translational effect of M-CSF. The increase in cholesterol ester synthesis was not a generalized effect, because there was no effect of acute incubation of M-CSF on metabolism of acetylated LDL. β-VLDL failed to stimulate cholesterol-ester synthesis in macrophages from LDL-receptor-deficient mice, either in the presence or absence of M-CSF. This indicated that the LDL receptor regulated both the basal and M-CSF effects of β-VLDL on cholesterol metabolism. The specific inhibitor of Gi/o, pertussis toxin, blocked the M-CSF-augmented β-VLDL-induced cholesterol esterification, but had no effect in the absence of the cytokine. Conversely, the effects of M-CSF on β-VLDL metabolism were mirrored by the direct Gi/o stimulant mastaparan.

Research into the mechanism of regulation of LDL receptors has previously been focused at the transcriptional level [6]. However, other studies have demonstrated post-line-translational regulation of LDL receptor [7], and the report of Whitman et al. [5*] provides a novel mode of regulation.

Tabas and coworkers [8] have provided extensive literature on the detrimental effects of intracellular accumulation of cholesterol, and the macrophage response to this deposition. Those investigators previously provided evidence that accumulation of unesterified cholesterol in macrophages is associated with activation of the major enzyme in the biosynthesis of phosphatidylcholine and of GTP:phosphocholine cytidylyltransferase-α (CTα), and with an accumulation of phosphatidylcholine [9]. This has been assumed to be a protective mechanism to attenuate unesterified cholesterol-induced cytotoxicity.

In order to test this hypothesis directly, Zhang et al. [10*] created mice in which CTα was selectively depleted in macrophages, and cell viability was determined during loading of unesterified cholesterol. Macrophage-specific depletion was accomplished using the Cre-lox system, in which mice that express Cre under the control of the endogenous M lysozyme promoter were bred with mice in which loxP sites were introduced between exons 4 and 5. One initial surprising result in these mice was that phosphatidylcholine biosynthesis was not absent as predicted, although it was decreased by over 70%. This is probably due to the activity of
CT/β2, which is a newly described enzyme in phosphatidylcholine biosynthesis that is present on a distinct gene locus relative to CTβ. The presence of this alternative pathway was probably serendipitous, because the complete absence of phosphatidylcholine biosynthesis would probably have negated the production of macrophages. In order to examine the effect of diminished phosphatidylcholine biosynthesis on macrophage viability, cells isolated from the peritoneum were loaded with unesterified cholesterol by incubation with acetylated LDL and the inhibitor of acyl coenzyme A-cholesterol acyltransferase, Sandoz 58035 [10⁷]. After 5 h there was a striking decrease in cell viability in the CTβ depleted macrophages as compared with control cells, as demonstrated by increased staining of Alexa-488 and propidium iodide. This decreased viability was not due to a generalized compromise of cell integrity, and was gauged by the response to the cell toxin staurosporine. That study is a striking demonstration of the use of molecular genetics to define a specific mechanistic pathway.

The ability of HDL to remove cholesterol from macrophages has been known for more than 2 decades [11]. However, there is still a lack of consensus on the properties of the acceptor particles that are important determinants for sterol removal. More recently, the novel scavenger receptor class B type I (SR-BI) has been implicated in the regulation of cholesterol fluxes [12]. All of the currently available data are consistent with the phospholipid content of HDL being the important determinant of cholesterol flux. Yancey et al. [13] have directly examined this hypothesis by manipulating the phosphatidylcholine and sphingomyelin content of HDL, and defined its effect on SR-BI-mediated cholesterol flux. HDL2 was more effective than HDL3 in promoting SR-BI-mediated cholesterol efflux when normalized to protein, but was the same as HDL3 when normalized to phospholipid. Supplementation of HDL with phospholipid through incubation with multilamellar vesicles increased cholesterol efflux, whereas depletion of phospholipid by phospholipase A₂ decreased cholesterol efflux. This effect was not related to differences in binding of HDL to SR-BI. Therefore, these data demonstrate that factors that change HDL phospholipid content will have a profound effect on reverse cholesterol transport.

These recent reports illustrate the complexity of cellular lipoprotein metabolism. However, they also provide intellectual challenges and potential pharmaceutical targets that are directed at the ultimate goal of maintaining the sterol balance within the microdomain of the arterial wall.

References

Previous studies have demonstrated that a substantial portion of the in-vivo metabolism of advanced glycation products is via the liver sinusoidal endothelial cells. It has been demonstrated that deficiency of class A scavenger receptor decreases the recognition in macrophages. However, this study demonstrated that deficiency of this receptor in cultured liver sinusoidal endothelial cells had no effect on the metabolism of advanced glycation products. Surprisingly, deficiency of this receptor also had no effect on the metabolism of acetylated LDL by these cells.


This paper describes the use of microassay techniques to determine genes that are regulated by oxidized LDL in the human macrophage cell line THP-1. This investigation found 268 genes that were regulated by the presence of this modified lipoprotein.


This is a description of a novel post-translational mechanism for acutely regulating the activity of LDL receptors.


This is the first demonstration that increases in interferon-γ through exogenous delivery leads to augmentation of the development of atherosclerosis in apolipoprotein E−/− mice.


This paper demonstrates that the major property of HDL that mediates efflux of cholesterol through SR-BI was the content of phospholipid.


This paper describes impressive use of molecular genetics to deplete one of the major enzymes that is involved in biosynthesis of phosphatidylcholine. Mice were genetically engineered that had a macrophage-specific reduction in their ability to biosynthesize this phospholipid. Cultured macrophages from these mice had a greater death rate in response to increases in the cellular content of unesterified cholesterol.