Lipoprotein Trafficking in Vascular Cells

MOLECULAR TROJAN HORSES AND CELLULAR SABOTEURS

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Recent studies have contributed to our understanding of the role of vascular cell activation in those processes related to lipoprotein trafficking, cholesterol accumulation, and atherosclerosis. In this regard, the field of lipoprotein receptor regulation has undergone a veritable explosion to define the critical links between the initial retention of apoprotein B-containing lipoproteins within the arterial wall and changes in vascular cell signaling that lead to cholesterol accumulation. New evidence has demonstrated that lipid peroxidation products generated during arterial entrapment of low density lipoprotein (LDL) can elicit the generation of an array of proinflammatory molecules that may activate the atherosclerotic process. These molecules include monocyte-specific chemotactants and hematopoietic growth factors.

In addition, we now recognize that oxidized lipids can activate arterial cells to produce greater levels of cytokines, which, in turn, can initiate signals that can alter the cholesterol trafficking process. This minireview summarizes current concepts regarding the panoply of major signaling events that result from lipoprotein-receptor interactions with vascular cells.

Lipoprotein Receptors and Cholesterol Delivery

Cholesterol, a weak amphiphile (1), is transported in the form of lipoproteins. LDL serves as the principal carrier, and it provides an exogenous source of cholesterol and other cellular nutrients to hepatic and extrahepatic tissues through LDL receptor-mediated uptake. Alternatively, LDL entrapped in arteries can undergo diverse enzymatic and chemical modifications. It can also introduce into the cell a variety of lipophilic invaders such as lipid peroxidation products and cholesterol oxides that may irreversibly modify cellular functions. Early stages of arterial lipoprotein modification, marked by generation of bioactive products of lipid peroxidation, can occur with little change in cellular receptor recognition. Such minimally oxidized LDL particles are molecular “Trojan horses,” physically similar to native plasma LDL but bearing a cargo of bioactive macromolecules. Subsequent stages of arterial lipoprotein modification include the generation of chemically reactive products of lipid peroxidation and aldehydic products such as malondialdehyde and 4-hydroxynonenal. They can induce covalent modification of LDL. Such highly oxidized LDL particles escape detection by LDL receptors and trigger recognition by several host-surveillance families of macrophage receptors. The uptake of these “cellular saboteurs” not only produces internalization of bioactive macromolecules but also facilitates the intracellular accumulation of lipoprotein-derived cholesterol and cholesteryl esters (CE) characteristic of arterial “foam” cell formation. The products of lipid peroxidation borne by molecular Trojan horses and cellular saboteurs trigger inflammatory reactions in vascular cells in vitro, causing the synthesis and release of chemokines and cytokines (Fig. 1). Proinflammatory cytokines, through up-regulation of gene encoding receptors for LDL and for modified LDL, can predispose vascular cells to accelerated internalization and accumulation of lipoprotein-derived lipids.

Delivery of cholesterol to vascular cells is mediated by several receptors. The high affinity receptor for LDL has been structurally and functionally characterized in detail and reviewed elsewhere (2). This receptor, known as the apoE receptor or the LDL receptor (LDL-R), is a ubiquitous cell surface transmembrane protein (3). Another important receptor is the apoE receptor found principally in hepatic cells and macrophages. It is mainly responsible for the binding and internalization of chylomicron remnants as well as apoE-containing LDL. The latter is a component of reverse cholesterol transport, i.e. delivery of cholesterol from extracellular tissue back to the liver. In addition, there is a receptor known as LDL-R-related protein, which can bind apoE-containing chylomicron-like remnants. Cholesteryl esters can enter the cell by any of these receptors, and they are metabolized through the CE cycle. This cycle has been extensively reviewed elsewhere (2, 4).

Scavenger receptors, also implicated in the accumulation of lipoprotein-derived CE in atherosclerotic lesions, are proteins that mediate the endocytosis of a diverse group of polyanions. Three classes of cloned scavenger receptors have recently been described (5). The first class, Class A receptors, includes the type I and II macrophage scavenger receptors (SR-AI and SR-AII). They are found predominantly on macrophages (6) and “activated” smooth muscle cells (7). SR-AI and SR-AII are homotrimeric membrane proteins, which are derived from alternatively spliced mRNA products of a single gene. Ligands for class A receptors include acetylated LDL, oxidized LDL, fucoidan, and carrageenan. The second class, Class B scavenger receptors, includes CD36 and SR-BI, which are found in adipose tissue (8), lung (8), liver (8), and macrophages (9). These receptors bind oxidized LDL, apoptotic cells, and anionic phospholipids (9). Recently, it has been shown that CD36 is expressed to a significant extent on atherosclerotic foam cells in human coronary arteries. A third class of scavenger receptors has been reported recently by Steinberg and colleagues (10) to be macroladin/C6D8, a family of endosomal proteins with sequence homology similar to the lysosomal-associated membrane proteins. The role of these lysosomal-associated membrane proteins in the uptake of oxidized LDL during atherogenesis remains to be elucidated.

Regulation of the LDL and Scavenger Receptors and Their Biological Impact

The major cell surface receptor for native LDL is the LDL-R. Regulation of LDL-R expression occurs primarily at the transcriptional level and is controlled by levels of free cholesterol in the cell. Inflammatory mediators such as growth factors and cytokines can promote the binding and uptake of LDL. These mediators include PDGF (11), TGF-β (12), basic fibroblast growth factor (13), TNF-α (14), and IL-1 (14) (Table I). Some of these mediators, such as TNF-α and IL-1, affect transcriptional regulation of the LDL-R gene at the level of the promoter (14). However, it is unlikely that cytokine activation of the LDL-R can result in cellular cholesterol

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1 The abbreviations used are: LDL, low density lipoprotein; CE, cholesteryl ester; HDL, high density lipoprotein; 4-HNE, 4-hydroxynonenal; HODE, hydroxyoctadecadienoic acid; IFN-γ, interferon-γ; IL-1, interleukin-1; LDL-R, low density lipoprotein receptor; mm-LDL, minimally modified low density lipoprotein; M-CSF, monocyte colony-stimulating factor; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PKC, protein kinase C; SR-A, scavenger receptor, class A; SR-B, scavenger receptor, class B; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; ICAM-1, intercellular adhesion molecule; VCAM-1, vascular cell adhesion molecule-1.
accumulation since TNF-α and IL-1 cannot override the inhibition of LDL-R activity by exogenous cholesterol (14).

Addition of LDL to vascular cells evokes a response reminiscent of classical signaling receptors. For example, LDL is mitogenic for both arterial endothelial and smooth muscle cells (15). Early studies suggested that the “mitogenic” effects of LDL were nutritional, resulting from the provision of cholesterol and essential fatty acids (arachidonate and linoleate) needed for cell growth and function (16). LDL can also directly stimulate PDGF production in endothelial cells (17).

It has been proposed that the atherosclerotic lesions develop, in part, by exposure of the arterial wall to minimally and oxidatively modified lipoproteins (18, 19). The biological properties of minimally modified LDL (mm-LDL) are quite distinct from those of native LDL or highly oxidized LDL. These molecular Trojan horses, like plasma LDL, can bind to the LDL-R but, unlike native LDL, bear a cargo of biologically active products of lipid peroxidation (20). The cellular acquisition of bioactive lipids induces endothelial cell responses predisposing to atherogenesis, and these include the ability to induce expression of monocyte-specific adhesion molecules, expression of the monocyte chemoattractant protein-1, synthesis of monocyte colony-stimulating factor (M-CSF), and tissue factor production (19). mm-LDL not only induces modulation of genes influencing inflammatory and vascular cell responses but also proteins involved in the defense against products of lipid peroxidation, e.g. glutathione transferase, heme oxidase, and inducible members of the serum amyloid A family (21). Moreover, mm-LDL can induce an inflammatory phenotype in endothelial cells accompanied by alterations in cellular signaling events. For example, the signaling mechanisms by which mm-LDL stimulates monocyte adhesion to endothelial cells are dependent on increased cyclic AMP formation since agonists that increase cyclic AMP can mimic the actions of mm-LDL. These agents also increase the levels of the transcription factor, NF-κB, which has been linked to the expression of a variety of adhesion molecules (22).

Highly oxidized LDL is readily distinguished from native and mm-LDL by chemical, biochemical, and cellular properties. Exposure of LDL to iron, copper, or other oxidants in vitro can lead to generation of metabolites from lipid peroxidation as well as covalent modification of apoB protein by chemically reactive, lipid aldehyde products (18). It has also been shown that vascular cells can markedly enhance the rate of LDL oxidation. Lysine aldehyde adducts, markers for highly oxidized LDL, have been demonstrated in macrophage-rich arterial lesions (23). Lipoxynase metabolites such as 12-hydroxyeicosatetraenoic acid from the aspirin-insensitive arachidonic acid pathway can also lead to oxidation of LDL in cells. Chemical neutralization of 15% of the amino group of lysine residues by malondialdehyde, a common end product of lipid peroxidation, or by products of transition metal-induced oxidation (24) can convert LDL to an anionic ligand recognized by class A (24, 25) and class B scavenger receptors (26) on macrophages. These derivatizations of apoB protein concomitantly abolish lipoprotein interactions with the LDL-R. Studies have shown that anionic clusters (27), some of which are presented by the N terminus of apoB-100 protein (28), form binding sites recognized by the scavenger receptors. Since expression of scavenger receptors is not down-regulated by cholesterol, macrophages expressing scavenger receptors can internalize substantial quantities of CE from highly oxidized LDL.

**Role of Cytokines in Delivery of Lipoprotein-bound Cholesterol**

The uptake of oxidized LDL by macrophages can be significantly influenced by the state of differentiation of the cell and its exposure to cytokines (Table I). The expression of Class A and Class B scavenger receptors is increased as monocytes differentiate into macrophages. In addition, it has now been shown that macrophage Class A scavenger receptors are down-regulated by TNF-α (29), IFN-γ (30), TGF-β (31), and lipopolysaccharide (29) but up-regulated by M-CSF (32) (Table I). Therefore, there are specific cytokine effects on scavenger receptor activity. Expression of CD63, a Class B scavenger receptor, which is responsible for a significant quantity of oxidized LDL binding and uptake by macrophages in vitro (10), is also increased by M-CSF (33).

Finally, new data now show that oxidized LDL, once bound to specific scavenger receptors, can initiate cell signaling events in vascular cells (34). For example, oxidized LDL can stimulate phosphoinositide metabolism and calcium flux in a pertussis toxin-sensitive manner (35) as well as stimulate phospholipase D activity through a tyrosine kinase-dependent mechanism independent of

![Diagram](http://www.jbc.org/)

**Effect of cytokines on lipoprotein receptor activities and intracellular cholesterol metabolism**

The abbreviations used are: ACEH, acid cholesteryl ester hydrolase; NCEH, neutral cholesteryl ester hydrolase; bFGF, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

**Table I**

<table>
<thead>
<tr>
<th>Arterial cell</th>
<th>Cytokines and growth factors</th>
<th>Receptor/enzyme</th>
<th>Response to agonists</th>
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<td>Smooth muscle cells</td>
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<td>LDL-R, SR-AI, ACEH, NCEH</td>
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protein kinase C (36). This may induce the release of phosphatidic acid or arachidonic acid for eicosanoid production in the vessel wall. In addition, a portion of this activity may be mediated by the Class A scavenger receptor. Specific Class A scavenger receptor ligands stimulate macropage uronase action (37), IL-1 production (38), and expression (39) in a FRC-dependent manner. Increases in ICAM-1 expression lead to enhanced monocyte adhesion to the vessel wall. Thus, cellular binding of oxidized LDL to the vessel wall can lead to a series of proatherosclerotic events.

Lipoprotein-derived Products of Lipid Peroxidation: Gift Horse or Hostile Invader?

Oxidative modification of LDL in vitro generates a broad array of products of lipid peroxidation. Both the diversity of product formation and observed cellular responses depend on the method and timing of oxidation (39). Propagation of these effects is complex, characterized by radical chain peroxidation of polyunsaturated fatty acids to lipid hydroperoxides, generation of cholsterol oxides and oxysterols, production of lysophosphatidylcholine, and generation of reactive aldehyde products of chemical decomposition. Moreover, certain products of lipid peroxidation generated during propagation are transient and undergo decomposition. The complexity of product generation and decomposition has stimulated basic investigative strategies to elucidate the proatherogenic complexity of product generation and decomposition. It has been shown that PAF acetylhydrolase can hydrolyze the bioactive lipids of mm-LDL and oxidized 1-palmitoyl-2-arachidonyl-sn-glycerophosphocholine (54). Recently, Watson et al. (54) have determined the molecular structure of two bioactive lipids through analyses of the isolated lipid fractions by electrospray ionization mass spectrometry. The lipids were identified as 1-palmitoyl-2-(5-oxovaleryl)-sn-glycerol-3-phosphocholine (m/z 594.3) and 1-palmitoyl-2-glutathionyl-sn-glycerol-3-phosphocholine (m/z 610.2). The third lipid (m/z 831) has tentatively been described as an arachidonic acid-containing phospho lipid containing three or four oxygen molecules, potentially forming a conjugated triene structure characteristic of leukotrienes (54). Watson et al. (54) have proposed that the latter may serve as a substrate for paraoxonase, and those with fragmentation products such as 5-oxovalerate at the sn-2 position may represent substrates for PAF acetylhydrolase. Evidence that these oxidized phospholipids may play a role in atherosclerosis was suggested by their presence in fatty streak lesions from cholesterol-fed rabbits and their macrophage/vascular cell/cytokine reactivity with natural antibod ies present in ApoE null mice (54).

In summary, the cytotoxicity of oxidized LDL may result from its binding to scavenger receptors and by non-receptor-mediated mechanisms. Future work using null deletion (knock-out) mice will facilitate an understanding of the role of scavenger receptor(s) in cell signaling. It remains to be determined how oxidized LDL and its constituent lipids activate vascular cells to induce a prothrombotic phenotype (presumably by activating the coagulation cascade), promote cytokine production linked with inflammation, augment arterial CE accumulation, and induce apoptosis.

Modulation of LDL Oxidation by HDL

Cholesterol efflux from arterial cells is mediated by HDL through adsorption of cell membrane cholesterol at the cell surface (55). HDL also promotes cholesterol efflux by a receptor-dependent mechanism through its apoA-I and apoA-II moieties. Studies of the apoA genes in null deletion mice have shown that HDL plays a significant role in the reduction of cholesterol from atherosclerotic lesions (56). HDL may exert anti-atherosclerotic protective effects by two independent mechanisms. First, HDL may interact with a cell surface receptor to promote net cholesterol removal through a PCK mechanism (57). Oram and colleagues (57) described a 110-kDa protein with the binding attributes of a bona fide receptor. In 1996, a second putative HDL binding protein of 82 kDa was identified as a member of the Class B scavenger receptor family, SR-B1 (58). This receptor was localized in hepatic and steroidogenic tissue where HDL functions as a cholesterol donor. However, it is unclear whether this receptor is involved in the uptake of HDL by liver cells or tissue. Whether HDL functions as a cholesterol acceptor and whether this novel HDL-binding protein mediates cholesterol efflux from arterial cells is not known. In addition, HDL has also been shown to be mitogenic for endothelial cells (16) and smooth muscle cells (59), presumably by stimulating c-myc expression (60). These studies provide a link to oncogenic proliferation in a variety of cell systems.

A second mechanism to explain the anti-atherogenic role of HDL has been provided by Fogelman and colleagues (19–22). They showed that enzymes associated with HDL, paraoxonase and PAF acetylhydrolase, can hydrolyze the bioactive lipids of mm-LDL and
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acute phase reactants, after cardiac surgery in human subjects. The acquisition of major lase. However, this protective effect of HDL diminishes upon in-

duced by mm-LDL. Thus, HDL may function to protect LDL thereby ablate monocyte adherence and endothelial activation in-

tracellular matrix can lead to the progressive oxidation of LDL because of the action of lipoxigenases, reactive oxygen species, peroxyni-

trite, and/or myeloperoxidase (62). A range of oxidized LDL species is thus generated, ultimately resulting in their delivery to vascular cells through several families of scavenger receptors (Fig. 1). These molecular Trojan horses and cellular saboteurs once formed or deposited in the cell can contribute to, and participate in, formation of macrophage- and smooth muscle-derived foam cells. A lipid-enriched fatty streak along the vessel wall can ensue. In addition to foam cell development, products of LDL peroxidation may activate endothelial cells, increase smooth muscle mitogenesis (63), or in-

duce apoptosis because of the effects of oxysterols and products of lipid peroxidation (64) (Fig. 1). Because antioxidant defenses may be limited in the microenvironment of the cell or within LDL, the oxidation process continues to progress. Enzymes associated with HDL such as PAF acetylhydrolase and paraoxonase can participate in the elimination of biologically active lipids, but diminished cellu-

lar antioxidant activity coupled with low levels of LDL may allow acceleration of the clinical course of vascular disease (19).

There is still much to be learned about how modified LDL ini-
tiate cellular signals that lead to inflammation, mitosis, or choles-
	erol accumulation. The present challenges include elucidation of the key signaling events that regulate lipoprotein-derived choles-
	erol trafficking in the vessel wall, which can impact on the patho-

genesis of vascular disease.

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REFERENCES


