Identification, Regulation and Function of a Novel Lectin-Like Oxidized Low-Density Lipoprotein Receptor

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Oxidatively modified low-density lipoprotein (ox-LDL) leads to endothelial activation, dysfunction and injury. Recently, a novel lectin-like receptor for ox-LDL (LOX-1) has been identified, primarily in the endothelial cells, and it allows uptake of ox-LDL into endothelial cells. This receptor is transcriptionally upregulated by tumor necrosis factor-alpha, angiotensin II, shear stress and ox-LDL itself. The expression of this receptor activates a variety of intracellular processes that lead to expression of adhesion molecules and endothelial activation. This receptor is highly expressed in the blood vessels of animals and humans with hypertension, diabetes mellitus and atherosclerosis. Expression of this receptor may also be relevant in intra-arterial thrombogenesis and myocardial ischemia-reperfusion injury. Identification and regulation of this receptor and understanding of signal transduction pathways may lead to new therapies of diseases characterized by endothelial dysfunction. (J Am Coll Cardiol 2002;39:1429–35) © 2002 by the American College of Cardiology Foundation

Oxidative modification of low-density lipoprotein (LDL) is a key step in the pathogenesis of atherosclerosis. Oxidized LDL (ox-LDL), through a variety of scavenger receptors (SR), such as SR-AI/II, CD36, SR-BI, macrosialin/CD68 and SREC, is taken up by monocytes and macrophages and smooth muscle cells and exerts its pro-atherogenic effects on the vessel wall (1,2). The classic SRs are absent or present in very small amounts in endothelial cells (3). However, it has long been suggested that endothelial cells internalize and degrade the modified form of LDL, including ox-LDL, by cell-surface receptors (4–6).

Oxidized LDL leads to endothelial activation, dysfunction and injury. Endothelial activation is believed to be a very early step in the evolution of atherosclerosis. Activation of endothelial cells results in expression of a variety of genes, such as endothelin, tissue factor, cyclo-oxygenase, nitric oxide synthase (NOS), growth factors and monocyte chemoattractant protein-1 (MCP-1). It also leads to expression of adhesion molecules to which inflammatory cells attach, followed by a cascade of events, including cell rolling, separation of the intercellular junction and subendothelial migration of inflammatory cells (7–12). Oxidized LDL also induces apoptosis in endothelial cells (13).

How endothelial cells endocytose ox-LDL is a subject of intense investigation. In 1997, Sawamura et al. (14) identified a lectin-like receptor for ox-LDL (LOX-1) in bovine aortic endothelial cells. Since then, a number of groups have confirmed the presence of this specialized receptor and have defined its regulation. A definition and understanding of the regulation of this receptor allows the opportunity to inter-

vene at endothelial phase in disease states in which ox-LDL plays a central role.

Lectin-like ox-LDL receptor is a type II membrane protein ($\sim$50 kDa) with a C-type lectin-like extracellular domain and a short cytoplasmic tail. Lectin-like ox-LDL receptor is present in the endothelium of human coronary arteries, rabbit and rat aortae and bovine endothelial cells (14–18). A small amount of LOX-1 has also been identified in macrophages, platelets and smooth muscle cells (19–21).

In human coronary artery endothelial cells (HCAECs), there is a single class of LOX-1 receptors with a B_{max} of $\sim$30 ng/mg protein and K_D of 1.7×10^{-8} mol/l, as determined by radioligand-binding studies (16,17). An excess of unlabeled ox-LDL inhibits iodine-125 labeled ox-LDL binding to LOX-1, and native LDL has no effect.

Activation of LOX-1 can be blocked in a nonspecific manner by polyinosinic acid and carrageenan, but not by the SR blocker fucoidin (16). Recently, a specific antisense phosphorothioate oligodeoxynucleotide directed at the 5′-coding sequence of human LOX-1 messenger ribonucleic acid (mRNA) has been designed (22,23). This antisense blocks the expression of LOX-1 in response to ox-LDL. Antibodies to the ox-LDL receptor have also been developed (15).

Activators of LOX-1. LOX-1 AND ITS LIGANDS. Endothelial activation or dysfunction elicited by ox-LDL and its lipid constituents has been shown to play a crucial role in the pathogenesis of atherosclerosis. Lectin-like ox-LDL receptor was identified as the major receptor for ox-LDL in endothelial cells. This receptor can support binding, internalization and proteolytic degradation of ox-LDL, but not significant amounts of acetylated LDL, which is a well-known, high-affinity ligand for class A SRs expressed by endothelial cells (SR-EC) (14,16).

A recent study by Chen et al. (24) showed that the lectin-like domain of LOX-1 is essential for ligand binding, but the neck domain is not. In particular, the large loop

Abbreviations and Acronyms

AT_1	= angiotensin II type 1
(e)NOS	= (endothelial) nitric oxide synthase
HCAEC	= human coronary artery endothelial cells
LDL	= low-density lipoprotein
LOX-1	= lectin-like oxidized low-density lipoprotein receptor
MAPK	= mitogen-activated protein kinase
MCP-1	= monocyte chemoattractant protein-1
ox-LDL	= oxidized low-density lipoprotein
RAS	= renin-angiotensin system
ROS	= reactive oxygen species
TNF-alpha	= tumor necrosis factor-alpha

between the third and fourth cysteine of the lectin-like domain plays a crucial role for ox-LDL binding, as well as C-terminal end-residues. Alanine-directed mutagenesis of the basic amino acid residues around this region revealed that all of the basic residues are involved in ox-LDL binding. Simultaneous mutations of these basic residues almost abolished the ox-LDL-binding activity of LOX-1. An electrostatic interaction between basic residues in the lectin-like domain of LOX-1 and negatively charged ox-LDL is critical for the binding activity of LOX-1.

Moriwaki et al. (25) reported that LOX-1 binds and degrades ox-LDL, but not acetylated LDL. Fucoidin and maleylated bovine serum albumin, which inhibit ox-LDL binding to class A SRs, do not inhibit ox-LDL binding or degradation in LOX-1–expressing cells (LOX-1-CHO). Polyinosinic acid and carrageenan, in contrast, significantly reduce ox-LDL binding to LOX-1-CHO. Oxidized LDL and delipidated and untreated LDL bind and are degraded similarly in LOX-1-CHO. Taken together, these observations show that LOX-1 is a receptor for ox-LDL, but not for acetylated LDL. LOX-1 recognizes protein moiety of ox-LDL, and its ligand specificity is distinct from that of other receptors for ox-LDL, including class A and B scavenger receptors (25). It is not known whether oxidized phospholipids and minimally oxidized LDL bind to LOX-1.

OXIDIZED LDL AND LYSOPHOSPHOTIDYLCHOLINE. Mehta and Li (16) and later on Aoyama et al. (26) described upregulation of LOX-1 mRNA in cultured endothelial cells by ox-LDL in a time-dependent fashion, with peak at 12 to 24 h of incubation in vitro. This effect of ox-LDL was concentration-dependent (10 to 40 μg/ml), with a decline at higher concentrations, probably a reflection of cell death at higher concentrations (80 to 100 μg/ml) (13). In contrast to ox-LDL, incubation of cells with native-LDL had no effect on the expression of LOX-1. The induction of LOX-1 protein is associated with an increase in mRNA, suggesting transcriptional upregulation of LOX-1 by ox-LDL. The upregulation of LOX-1 in response to ox-LDL can be blocked by a specific antisense to LOX-1 mRNA (22,23).

Lysophosphotidylcholine, which has been implicated in atherogenesis, also induces mRNA and protein expression of LOX-1 (26). Because lysophosphotidylcholine does not change the half-life of LOX-1 mRNA, the upregulation of LOX-1 appears to occur at transcriptional levels.

TUMOR NECROSIS FACTOR-ALPHA (TNF-ALPHA). The cytokine TNF-alpha is a pro-inflammatory cytokine. Its expression is increased in atherosclerosis, and excess production of TNF-alpha influences plaque vulnerability (27–29). Kume et al. (30) showed that TNF-alpha increases LOX-1 cell-surface expression in a concentration-dependent manner, with a peak time to expression of 8 to 12 h. Tumor necrosis factor-alpha appeared to activate the transcription of LOX-1, as measured by nuclear run-off assay. Another cytokine, interferon-gamma, however, does not increase the expression of LOX-1. It is noteworthy that TNF-alpha can induce the expression of class A SRs in cultured vascular smooth muscle cells (31,32), but it suppresses the expression of class A SRs in macrophages (33).

FLUID SHEAR STRESS. Shear stress modulates the expression of various genes in endothelial cells and is an important determinant of endothelial cell function. Shear stress in the physiologic range (1 to 15 dynes/cm^2) was shown by Murase et al. (34) to upregulate LOX-1 mRNA and protein in a time-dependent fashion, with a peak at 4 and 8 h, respectively. Chelation of intracellular Ca^{++} reduced shear stress-induced LOX-1 mRNA expression, and the Ca^{++} ionophore ionomycin enhanced LOX-1 mRNA expression.

Upregulation of LOX-1 expression in response to fluid shear stress may be important in endothelial cell injury and activation.

ANGIOTENSIN II. Activation of the renin-angiotensin system (RAS), resulting in the formation of angiotensin II, is a critical factor in atherogenesis. Inhibition of RAS by the angiotensin-converting enzyme has been shown to be effective in limiting atherosclerosis (35) and decreasing cardiac events associated with atherosclerosis (36). Most of the effects of angiotensin II are thought to be mediated by activation of the angiotensin II type 1 (AT_1) receptor. Angiotensin II, like ox-LDL, causes endothelial apoptosis, decreases NOS expression, stimulates reactive oxygen species (ROS) formation and induces expression of adhesion molecules in endothelial cells (37–40).

Angiotensin II markedly increases LOX-1 mRNA and protein expression (17). The increase in LOX-1 expression occurs in a concentration-dependent manner. Angiotensin II causes an increase in ox-LDL uptake by endothelial cells, again in a concentration-dependent manner. Angiotensin II causes endothelial cell injury and potentiates ox-LDL–mediated cell injury. Most importantly, these effects of angiotensin II can be blocked by specific AT_1 receptor blockers, but not by AT_2 receptor blockers. Other studies suggest that ox-LDL upregulates angiotensin II AT_1 receptor expression (41). Several studies show that hypercholes-

terolemia is associated with enhanced AT_1 receptor expression (42,43).

These observations collectively imply the presence of the phenomenon of cross-talk between ox-LDL and RAS in atherosclerosis. Each of these mediators recruits the other, and together, the two systems cause extensive cell activation, dysfunction and injury.

LOX-1 and endothelial cell injury. A number of studies have shown that ox-LDL causes endothelial activation, dysfunction and injury (7,13,44). An important form of injury that has recently attracted attention is programmed cell death, or apoptosis. Recently, we demonstrated that ox-LDL causes apoptosis in HCAECs in association with upregulation of apoptotic protein Fas and downregulation of the anti-apoptotic protein Bcl2 (13). Endothelial cell apoptosis was characterized by fluorescent TUNEL staining and deoxyribonucleic acid laddering. Oxidized LDL-mediated apoptosis was associated with upregulation of LOX-1 expression. Importantly, two different chemical inhibitors of LOX-1, as well as a specific antisense to LOX-1 mRNA, decreased apoptosis (22). These inhibitors of LOX-1 activation decreased the release of lactic dehydrogenase, a marker of cell injury.

It is important to note that apoptosis is a common occurrence in atherosclerosis (44) and in a number of other atherosclerosis-related disease processes, such as myocardial ischemia (45).

A recent study (46) from our group demonstrated that ox-LDL decreases the expression of endothelial constitutive NOS (eNOS) and increases the expression of the following adhesion molecules in HCAECs: E- and P-selectins, intracellular adhesion molecule-1 and vascular cell adhesion molecule-1. Importantly, we found that LOX-1 plays a crucial role in this setting, because inhibition of LOX-1 by LOX-1 antisense reduces the effect of ox-LDL on the expression of eNOS and adhesion molecules. These observations, taken together, indicate that LOX-1 plays a major role in endothelial dysfunction and injury, leading to the initiation and progression of atherosclerosis.

Oxidized LDL has also been well known to play a key role in the adherence of monocytes to the activated endothelium, an early step in atherogenesis (7). The intracellular processes involved include activation of protein kinase C and mitogen-activated protein kinase (MAPK) (47), as well as the subsequent upregulation of MCP-1 (47–49). In studies of HCAECs (23), we found that incubation of endothelial cells with ox-LDL increased the phosphorylation of MAPK. In these experiments, ox-LDL also upregulated MCP-1 expression (protein and mRNA) and monocyte adhesion to the endothelial cells through activation of LOX-1. The definitive evidence for the hypothesis that LOX-1 induces upregulation of MCP-1, with subsequent adhesion of monocytes to endothelial cells, through LOX-1 activation with activation of MAPK as a key signal transduction mechanism, came from experiments in which the antisense to LOX-1 mRNA completely blocked these processes in endothelial cells treated with ox-LDL.

LOX-1 AND SIGNALING PATHWAYS. The signaling pathways for ox-LDL–mediated endothelial injury include the intracellularly generated ROS and activation of protein kinases and transcription factors (50–54). Cominacini et al. (49) showed that it is the binding of ox-LDL to LOX-1 that initiates nuclear factor-κB activation, as well as the increase in intracellular ROS formation. These effects of ox-LDL were blocked by a monoclonal antibody to LOX-1. Direct evidence for ox-LDL–mediated intracellular ROS formation in endothelial cells through activation of LOX-1 has recently been presented (50).

As mentioned earlier, treatment of HCAECs with ox-LDL resulted in the activation of MAPK and transcriptional nuclear factor-κB, and subsequently expression of several genes (22). Further experiments showed that the antisense to LOX-1 mRNA inhibited these signal transduction pathways elicited by ox-LDL (22).

A recent study (55) showed that LOX-1 expression is associated with inactivation of the protein kinase B pathway. Protein kinase B, the cellular homologue of v-Akt, is a key signaling component downstream of phosphatidylinositide-3 kinase (56). Activation of protein kinase B appears to be vitally important in the expression of eNOS.

Upregulation of LOX-1, the signal conduction pathways in its biologic effects and the role of LOX-1 in steps leading to cell injury and ultimately atherosclerosis are summarized in Figure 1.

Role of LOX-1 in disease states. Oxidized LDL has been shown to activate endothelial cells as well as platelets. Oxidized LDL decreases eNOS synthesis in endothelial cells, platelets and leukocytes (57–59). By immunostaining, ox-LDL has been identified in atherosclerotic plaque, particularly rupture-prone plaque (60). In addition, plasma levels of ox-LDL are elevated in patients with acute manifestations of atherosclerosis (60). Oxidized LDL has been shown to upregulate the expression of another local mediator of atherogenesis—that is, the AT_1 receptor (41). Oxidized LDL also enhances the cell-injurious effects of angiotensin II, and angiotensin II, in turn, enhances the expression of LOX-1 and, thereby, the cell-injurious effects of ox-LDL (17). The crucial role of RAS activation has been well established in patients with hypertension and atherosclerosis. These disease states are associated with upregulation of LOX-1, which suggests a pathogenic role of this novel receptor, either alone or in combination with RAS activation.

HYPERTENSION. In studies by Nagase et al. (61), spontaneously hypertensive rats, Wistar-Kyoto rats and Dahl salt-sensitive and salt-resistant rats were fed a salt-loaded or control diet. Lectin-like ox-LDL receptor mRNA, as determined by Northern blot analysis, was found to be minimal in the aorta and veins of Wistar-Koyoto rats, but was markedly upregulated in spontaneously hypertensive
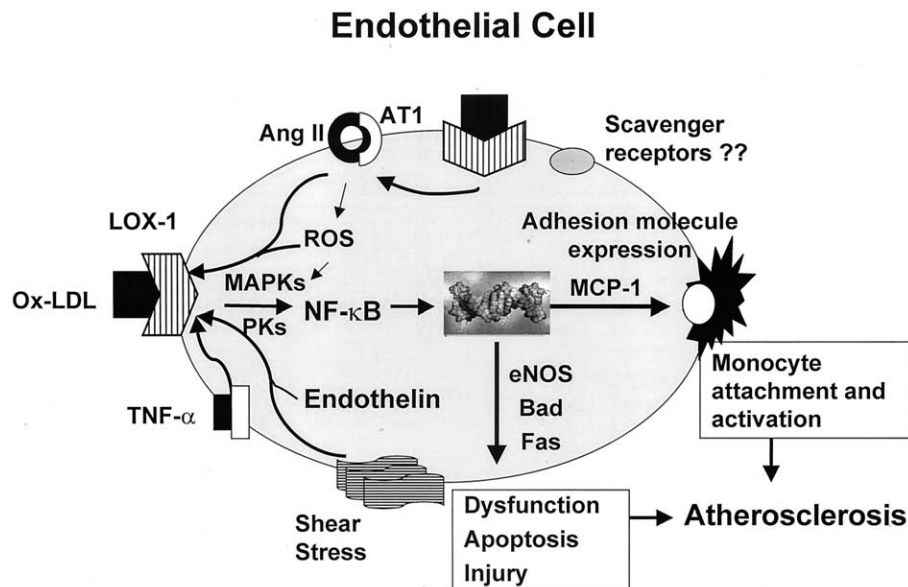
Endothelial Cell

Figure 1. Oxidized low-density lipoprotein (ox-LDL), tumor necrosis factor-alpha (TNF-α), shear stress, reactive oxygen species (ROS), endothelin and angiotensin II (Ang II) increase the expression of lectin-like oxidized low-density lipoprotein receptor (LOX-1), which activates mitogen-activated protein kinases (MAPKs) and protein kinases (PKs), leading to activation of the transcription factor nuclear factor-κB (NF-κB). These steps play a crucial role in subsequent cell injury, through release of ROS and a reduction in endothelial nitric oxide synthase (eNOS). Enhanced activity of monocyte chemoattractant protein-1 (MCP-1) leads to expression of adhesion molecules. Simultaneously, LOX-1 activation leads to endothelial dysfunction, apoptosis and injury. These steps are collectively involved in atherogenesis. Along with decreases in eNOS, changes in Fas and bad lead to apoptosis. AT_1 = angiotensin II type 1.

rats. Expression of LOX-1 was lower in the aorta of Dahl salt-resistant rats on both diets, but was higher in salt-loaded Dahl salt-sensitive rats. These investigators also suggested that LOX-1 expression in hypertensive rats might be dynamically regulated. This concept is further supported by the observations that angiotensin II upregulates LOX-1 gene expression in endothelial cells (17), and angiotensin-converting enzyme inhibitors markedly decrease LOX-1 gene expression (62). These findings suggest that LOX-1 expression is upregulated in hypertension and may contribute to its pathogenesis. It may be speculated that enhanced LOX-1 expression is the cause of increased ox-LDL uptake in endothelial cells, as well as diminished endothelium-dependent vasorelaxation in hypertension (63).

ATHEROSCLEROSIS. Chen et al. (15) showed LOX-1 expression (protein and mRNA) to be upregulated in the aortae of eight-week-old Watanabe-heritable hyperlipidemic rabbits. Immunostaining showed that the augmented expression was primarily localized within the intima at the early stages of atherosclerosis. The most prominent staining effect was observed in the endothelial cells of atherosclerotic lesions; however, the endothelium of the areas without lesions in the rabbits' aortae also demonstrated LOX-1 accumulation, suggesting that LOX-1 may be involved in the initiation of atherosclerosis.

We studied New Zealand White rabbits on a 10-week, high-cholesterol diet and observed upregulation of LOX-1 gene expression in the aortae of hypercholesterolemic rabbits, but not in the aortae of rabbits on a regular diet (64). Immunostaining showed that LOX-1 was present primarily

in the neointima. Importantly, treatment of rabbits with the AT_1 receptor blocker, losartan, not only decreased the extent of atherosclerosis, but also reduced LOX-1 expression (by reverse-transcription polymerase chain reaction and immunostaining). The vascular tissues in this model of atherosclerosis have previously been shown to over-express AT_1 receptors (43). These observations collectively provide in vivo evidence for cross-talk between LOX-1 accumulation and AT_1 receptor upregulation.

Kataoka et al. (65) examined human carotid artery endarterectomy specimens and showed positivity for LOX-1 expression in early atherosclerotic lesions, and the positivity was greater in recent compared with advanced lesions. They found that besides endothelial cells, macrophages and smooth muscle cells in the intima of advanced atherosclerotic plaques demonstrated LOX-1 positivity. In our laboratory, we have identified LOX-1 expression in the intima of the microvasculature of advanced human atherosclerotic lesions. Furthermore, we observed co-localization of LOX-1 in apoptotic cells on double immunostaining. The cells expressing apoptosis and LOX-1 were endothelial cells, smooth muscle cells and macrophages. Notably, Oka et al. (66) have shown that LOX-1 mediates phagocytosis of aged and apoptotic cells. These observations in atherosclerotic tissues collectively confirm the results of in vitro studies (13), and suggest that LOX-1 expression in the early stages of atherosclerosis may serve to facilitate the uptake of ox-LDL and activate endothelial cells to which inflammatory cells adhere. As the process of atherogenesis progresses, LOX-1 is expressed in the smooth muscle cells and mono-

cytes and macrophages, where, in conjunction with expression of classic scavenger receptors, LOX-1 may serve to induce apoptosis and phagocytosis.

THROMBOSIS. Thrombosis is usually the early event that leads to acute manifestations of atherosclerosis, and platelets are the usual initiator cells in this process (67). Platelets have been shown to internalize ox-LDL, which serves to decrease type 3 NOS (eNOS) activity in platelets and enhance platelet aggregation (58). Recently, platelets have also been shown to express a modest amount of LOX-1 (19). Kakatani et al. (68), in preliminary studies, have shown that LOX-1 antibody decreases arterial thrombus formation in the rat. This observation provides a link between internalization of ox-LDL and platelet activation and thrombosis.

MYOCARDIAL ISCHEMIA. Thrombosis formed in the coronary arteries is often the most proximal cause of acute coronary syndromes (67). Oxidized LDL is present in the atherosclerotic tissues of rupture-prone segments (60). Furthermore, the degree of myocardial ischemia is characterized by the oxidative state, which facilitates oxidation of native LDL (60). An in vitro study showed that perfusion of ox-LDL significantly decreases myocardial contraction in the isolated heart of the rat in the Langendorff model (69). We recently examined LOX-1 expression in the myocardium of rats undergoing total coronary occlusion for 1 h, followed by reperfusion for 2 h. In these rat hearts, we observed extensive LOX-1 expression in the ischemic-reperfused myocardium, but not in the hearts of rats subjected to a sham operation. Ischemia alone did not cause a marked upregulation of LOX-1 expression. To examine the contributory role of LOX-1 in the determination of infarct size and cardiac function, a group of rats was treated with LOX-1 antibody before initiation of ischemia. We observed a marked decrease in the expression of LOX-1 in the myocardium of rats given the antibody. Further, there was 48% reduction in infarct size, as well as preservation of left ventricular function after ischemia-reperfusion in rats given the LOX-1 antibody.

These studies imply LOX-1's important role during myocardial ischemia, in terms of determination of cardiac function and infarct size.

DIABETES MELLITUS. Diabetes is characterized by a state of oxidative stress, endothelial dysfunction and upregulated expression of adhesion molecules in inflammatory cells. In a rat model of diabetes in response to streptozocin, LOX-1 expression was found to be increased in the aorta of the diabetic rat, compared with that of the nondiabetic rat (70). Immunohistochemistry revealed that the most distinctive staining effect of LOX-1 was in the endothelial cells, especially in the region of bifurcation of the aorta. Furthermore, in cultured aortic endothelial cells, diabetic rat serum and advanced glycosylation end-products induced LOX-1 expression, whereas control rat serum, along with high glucose levels, did not. In addition, with a competitive inhibition assay, LOX-1 ligand activity was accumulated in the diabetic rat serum, mainly in the very-low-density lipoprotein/LDL fractions.

Conclusions. Identification of LOX-1 and a definition of its biologic role in pathophysiologic states provide a new clue to the reason for uptake of ox-LDL in endothelial cells. Internalization of ox-LDL leads to a cascade of events that may induce a variety of diseases characterized by endothelial dysfunction, activation and injury. Activation of endothelial cells by ox-LDL through LOX-1 upregulation may be a key event in hypertension, diabetes mellitus and dyslipidemia—the most important risk factors for atherosclerosis. Cross-talk between different systems, such as RAS and dyslipidemia, may serve to enhance tissue injury.

A definition of the underlying signals leading to inflammatory cell deposits and activation provides an opportunity to understand the disease process and to design novel therapies in the management of atherosclerosis.

REFERENCES

1. Freeman MW. Scavenger receptors in atherosclerosis. Curr Opin Hematol 1997;4:41–7.
2. Steinbrecher UP. Receptors for oxidized low density lipoprotein. Biochem Biophys Acta 1999;1436:279–98.
3. Bickel PE, Freeman M. Rabbit aortic smooth muscle cells express inducible macrophage scavenger receptor messenger RNA that is absent from endothelial cells. J Clin Invest 1992;90:1450–7.
4. Kume N, Arai H, Kawai C, et al. Receptors for modified low-density lipoproteins in human endothelials cells: different recognition for acetylated low-density lipoprotein and oxidized low-density lipoprotein. Biochem Biophys Acta 1991;1091:63–7.
5. van Berkel TJC, De Rijke YB, Kruijt JK. Different fate in vivo of oxidatively modified low density lipoprotein and acetylated low density lipoprotein in rats. J Biol Chem 1991;266:2282–9.
6. De Rijke YB, van Berkel TJC. Rat liver Kupffer and endothelial cells express different binding proteins for modified low density lipoproteins. J Biol Chem 1994;269:824–7.
7. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–9.
8. Kume N, Cybulsky MI, Gimbrone MA, Jr. Lysophosphatidylcholinea component of atherogenic lipoproteins induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J Clin Invest 1992;90:1138–44.
9. Kume N, Gimbrone MA, Jr. Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. J Clin Invest 1994;93:907–11.
10. Zembowicz A, Tang JL, Wu KK. Transcriptional induction of endothelial nitric oxide synthase type III by lysophosphatidylcholine. J Biol Chem 1995;270:17006–10.
11. Hirata K, Miki N, Kuroda Y, et al. Low concentration of oxidized low-density lipoprotein and lysophosphatidylcholine upregulate constitutive nitric oxide synthase mRNA expression in bovine aortic endothelial cells. Circ Res 1995;76:958–62.
12. Zembowicz A, Jones SL, Wu KK. Induction of cyclooxygenase-2 in human umbilical vein endothelial cells by lysophosphatidylcholine. J Clin Invest 1995;96:1688–92.
13. Li DY, Yang BC, Mehta JL. Ox-LDL enhances anoxia-reoxygenation-mediated apoptosis in human coronary endothelial cells: role of PKC, PTK, Bcl-2 and Fas. Am J Physiol 1998;275:H568–76.

14. Sawamura T, Kume N, Aoyama T, et al. An endothelial receptor for oxidized low-density lipoprotein. Nature 1997;386:73–7.

15. Chen M, Kakutani M, Minami M, et al. Increased expression of lectin-like oxidized low density lipoprotein receptor-1 in initial atherosclerotic lesions of Watanabe heritable hyperlipidemic rabbits. Arterioscler Thromb Vasc Biol 2000;20:1107–15.

16. Mehta JL, Li DY. Identification and autoregulation of receptor for Ox-LDL in cultured human coronary artery endothelial cells. Biochem Biophys Res Commun 1998;248:511–4.

17. Li DY, Zhang YC, Philips MI, et al. Upregulation of endothelial receptor for oxidized low-density lipoprotein (LOX-1) in cultured human coronary artery endothelial cells by angiotensin II type 1 receptor activation. Circ Res 1999;84:1043–9.

18. Nagase M, Hirose S, Sawamura T, et al. Enhanced expression of endothelial oxidized low-density lipoprotein receptor (LOX-1) in hypertensive rats. Biochem Biophys Res Commun 1997;237:496–8.

19. Chen M, Kakutani M, Naruko T, et al. Activation-dependent surface expression of LOX-1 in human platelets. Biochem Biophys Res Commun 2001;282:153–8.

20. Kataoka H, Kume N, Miyamoto S, et al. Oxidized LDL modulates Bax/Bcl-2 through the lectin-like Ox-LDL receptor-1 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2001;21:955–60.

21. Yoshida H, Kondratenko N, Green S, et al. Identification of the lectin-like receptor of oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. Biochem J 1998;334:9–13.

22. Li D, Mehta JL. Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells: evidence from use of antisense LOX-1 mRNA and chemical inhibitors. Arterioscler Thromb Vasc Biol 2000;20:1116–22.

23. Li D, Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. Circulation 2000;101:2889–95.

24. Chen M, Inoue K, Narumiya S, et al. Requirements of basic amino acid residues within the lectin-like domain of LOX-1 for the binding of oxidized low-density lipoprotein. FEBS Lett 2001;499:215–9.

25. Moriwaki H, Kume N, Sawamura T, et al. Ligand specificity of LOX-1, a novel endothelial receptor for oxidized low density lipoprotein. Arterioscler Thromb Vasc Biol 1998;18:1541–7.

26. Aoyama T, Fujiwara H, Masaki T, et al. Induction of lectin-like oxidized LDL receptor by oxidized LDL and lysophosphatidylcholine in cultured endothelial cells. J Mol Cell Cardiol 1999;31:2101–14.

27. Tipping PG, Hancock WW. Production of tumor necrosis factor and interleukin-1 by macrophages from human atheromatous plaques. Am J Pathol 1993;142:1721–8.

28. Barath P, Fishbein MC, Cao J, et al. Tumor necrosis factor gene expression in human vascular intimal smooth muscle cells detected by in situ hybridization. Am J Pathol 1990;137:503–9.

29. Lee RT, Libby P. The unstable atheroma. Arterioscler Thromb Vasc Biol 1997;17:1859–67.

30. Kume N, Murase T, Moriwaki H, et al. Inducible expression of lectin-like oxidized LDL receptor-1 in vascular endothelial cells. Circ Res 1998;83:322–7.

31. Pitas RE. Expression of the acetylated low density lipoprotein receptor by rabbit fibroblasts and smooth muscle cells: upregulation by phorbol esters. J Biol Chem 1990;265:12722–7.

32. Li H, Freeman MW, Libby P. Regulation of smooth muscle scavenger receptor expression in vivo by atherogenic diets and in vitro by cytokines. J Clin Invest 1995;95:122–33.

33. Hsu HY, Nicholson AC, Hajjar DP. Inhibition of macrophage scavenger activity by tumor necrosis factor-α is transcriptionally and post-transcriptionally regulated. J Biol Chem 1996;271:7767–73.

34. Murase T, Kume N, Korenaga R, et al. Fluid shear stress transcriptionally induces lectin-like oxidized LDL receptor-1 in vascular endothelial cells. Circ Res 1998;83:328–33.

35. Chobanian AV, Hauderschild CC, Nickerson C, et al. Trandolapril inhibits atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Hypertension 1992;20:473–7.

36. Pitt B. Angiotensin-converting enzyme inhibitors in patients with coronary atherosclerosis. Am Heart J 1994;128:1328–32.

37. Li DY, Yang BC, Phillips MI, et al. Proapoptotic effects of Ang II in human coronary artery endothelial cells: role of AT$_1$ receptor and PKC activation. Am J Physiol 1999;276:H786–92.

38. Raij L. Hypertension and cardiovascular risk factors: role of the angiotensin II–nitric oxide interaction. Hypertension 2001;37:767–73.

39. Lassegue B, Sorescu D, Szocs K, et al. Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res 2001;88:888–94.

40. Dzau VJ. Tissue angiotensin and pathobiology of vascular disease: a unifying hypothesis. Hypertension 2001;37:1047–52.

41. Li D, Saldeen T, Romeo F, et al. Oxidized LDL upregulates angiotensin II type 1 receptor expression in cultured human coronary artery endothelial cells: the potential role of transcription factor NF-κB. Circulation 2000;102:1970–6.

42. Strehlow K, Wassmann S, Bohm M, et al. Angiotensin AT$_1$ receptor over-expression in hypercholesterolaemia. Ann Med 2000;32:386–9.

43. Yang BC, Phillips MI, Mohuczy D, et al. Increased angiotensin II type 1 receptor expression in hypercholesterolemic atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol 1998;18:1433–9.

44. Walsh K, Isner JM. Apoptosis in inflammatory-fibroproliferative disorders of the vessel wall. Cardiovasc Res 2000;45:756–65.

45. Sam F, Sawyer DB, Chang DL, et al. Progressive left ventricular remodeling and apoptosis late after myocardial infarction in mouse heart. Am J Physiol 2000;279:H422–8.

46. Mehta JL, Li DY, Chen HJ, Joseph J, Romeo F. Inhibition of LOX-1 by statins may relate to upregulation of eNOS. Biochem Biophys Res Commun 2001;289:857–61.

47. Voraberger G, Schafer R, Strotowa C. Cloning of the human gene for intercellular adhesion molecule-1 and analysis of its 5′-regulatory region: induction by cytokines and phorbol ester. J Immunol 1991;147:2777–86.

48. Iademarco MF, McQuillan JJ, Rosen GD, et al. Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). J Biol Chem 1992;267:16323–9.

49. Cominacini L, Pasini AF, Garbin U, et al. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-κB through an increased production of intracellular reactive oxygen species. J Biol Chem 2000;275:12633–8.

50. Cominacini L, Rigoni A, Pasini AF, et al. The binding of oxidized low density lipoprotein (ox-LDL) to ox-LDL receptor-1 reduces the intracellular concentration of nitric oxide in endothelial cells through an increased production of superoxide. J Biol Chem 2001;276:13750–5.

51. Li DY, Chen HJ, Romeo F, et al. LOX-1–mediated adhesion molecule expression in human coronary artery endothelial cells: modulation by statins (abstr). Circulation 2000;102:1229.

52. Kusuhara M, Chait A, Cader A, et al. Oxidized LDL stimulates mitogen-activated protein kinases in smooth muscle cells and macrophages. Arterioscler Thromb Vasc Biol 1997;17:141–8.

53. Han KH, Tangirala RK, Green SR, et al. Chemokine receptor CCR2 expression and monocyte chemoattractant protein-1–mediated chemotaxis in human monocytes: a regulatory role for plasma LDL. Arterioscler Thromb Vasc Biol 1998;18:1983–91.

54. Wang GP, Deng ZD, Nj J, et al. Oxidized low density lipoprotein and very low density lipoprotein enhance expression of monocyte chemoattractant protein-1 in rabbit peritoneal exudates macrophages. Atherosclerosis 1997;133:31–6.

55. Li DY, Chen HJ, Mehta JL. Statins inhibit oxidized-LDL–mediated LOX-1 expression, uptake of ox-LDL and reduction in PKB phosphorylation. Cardiovasc Res 2001;52:217–25.

56. Tang X, Downes CP, Whetton AD, et al. Role of phosphatidylinositol-3 kinase and specific protein kinase B isoforms in the suppression of apoptosis mediated by the Abelson protein-tyrosine kinase. J Biol Chem 2000;275:3142–8.

57. Liao JK, Shin WS, Lee WY, et al. Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. J Biol Chem 1995;270:319–24.

58. Chen LY, Mehta P, Mehta JL. Oxidized LDL decreases L-arginine uptake and nitric oxide synthase protein expression in human platelets: relevance of the effect of oxidized LDL on platelet function. Circulation 1996;93:1740–6.

59. Mehta JL, Bryant JL, Jr, Mehta P. Reduction of nitric oxide synthase activity in human neutrophils by oxidized low-density lipoproteins: reversal of the effect of oxidized low-density lipoproteins by high-density lipoproteins and L-arginine. Biochem Pharmacol 1995;50:1181–5.

60. Ehara S, Ueda M, Naruko T, et al. Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. Circulation 2001;103:1955–60.

61. Nagase M, Hirose S, Sawamura T, et al. Enhanced expression of endothelial oxidized low-density lipoprotein receptor (LOX-1) in hypertensive rats. Biochem Biophys Res Commun 1997;237:496–8.

62. Morawietz H, Roreckschloss U, Niemann B, et al. Angiotensin II induces LOX-1, the human endothelial receptor for oxidized low-density lipoprotein. Circulation 1999;100:899–902.

63. Panza JA, Quyyumi AA, Brush JEJ, et al. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990;323:22–7.

64. Chen H, Li D, Sawamura T, et al. Upregulation of LOX-1 expression in aorta of hypercholesterolemic rabbits: modulation by losartan. Biochem Biophys Res Commun 2000;276:1100–4.

65. Kataoka H, Kume N, Miyamoto S, et al. Expression of lectin-like oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. Circulation 1999;99:3110–7.

66. Oka K, Sawamura T, Kikuta K, et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. Proc Natl Acad Sci USA 1998;95:9535–40.

67. Conti CR, Mehta JL. Acute myocardial ischemia: role of atherosclerosis, in situ thrombosis, platelet activation, coronary vasospasm, and altered arachidonic acid metabolism. Circulation 1987;75 Suppl V:V84–95.

68. Kakatani M, Sawamura T, Chen M. Role of LOX-1 in thrombosis (abstr). Circulation 2000;102 Suppl II:II191.

69. Harrison GJ, Jordan LR, Selleg ML, et al. Low-density lipoproteins inhibit histamine and $NaNO_2$ relaxation of the coronary vasculature and reduce contractile function in isolated rat hears. Heart Vessels 1995;10:249–57.

70. Kita T, Kume N, Ishii K, et al. Oxidized LDL and expression of monocyte adhesion molecules. Diabetes Res Clin Pract 1999;45:123–6.