How Do Oxidized Phospholipids Inhibit LPS Signaling?

Nigel Mackman

In 1981, Henriksen and coworkers reported that oxidized LDL (oxLDL) induces cholesterol accumulation in macrophages. This observation formed the basis of the hypothesis that oxidation of LDL might be an important step in the atherogenesis process. Many subsequent studies support the oxidative modification hypothesis of atherogenesis. However, oxidation of LDL is a complex process. Both the protein and the lipid molecules of LDL can be oxidatively modified resulting in a variety of biologically active molecules. The primary targets of oxidation are the esterified polyunsaturated fatty acids in the phospholipid shell that surrounds the insoluble neutral lipids of the lipoprotein core (Figure 1A). Importantly, atherosclerotic lesions contain antigens recognized by antibodies generated against oxLDL, demonstrating the presence of oxLDL in vivo.

OxLDL has both stimulatory and inhibitory effects on gene expression in vascular cells, such as monocytes/macrophages, endothelial cells, and smooth muscle cells. Different lipid oxidation products, such as oxidized phospholipids, oxidized cholesterol esters, and isoprostanes, were found to activate endothelial cells. In addition, lysophosphatidic acid induced TF expression in smooth muscle cells. Other studies by Marathe and coworkers demonstrated that one of the phospholipid oxidation products of oxidized LDL was a platelet-activating factor (PAF)-like molecule that activated the PAF receptor on platelets and leukocytes. Cushing et al and Rajavashisth et al showed that minimally oxidized LDL (mmLDL) induced the expression of chemokines and cytokines by endothelial cells (Figure 1A). Another study showed that mmLDL induced tissue factor (TF) expression in endothelial cells. The effects of mmLDL were found to be mediated by a G protein–coupled receptor via a cAMP-dependent pathway, although the specific receptor has not yet been identified. Interestingly, the effects of oxidized phospholipids can be blocked by PAF receptor antagonists, suggesting that the receptor may be related to the PAF receptor.

Watson and coworkers isolated and partially characterized some bioactive oxidized phospholipids in mmLDL. Oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), which includes 1-palmitoyl-2(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine (POVPC) and 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine (PGPC), was present in mmLDL and activated endothelial cells. oxPAPC also was shown to activate the transcription factors PPARγ, Egr-1, and NFAT but not NF-κB, and induce the expression of the pro-atherogenic genes MCP-1, IL-8, and TF in endothelial cells (Figure 1A). oxPAPC induction of the IL-8 promoter was mediated by cis-acting DNA elements that were distinct from those required for induction by TNFα, indicating that these agonists activated different transcription factors.

Lipopolysaccharide (LPS), also known as endotoxin, is the principal component of the outer membrane of Gram-negative bacteria. In humans, the presence of LPS in the blood is detected by serum-binding proteins and cell surface receptors on monocytes and endothelial cells (Figure 1B). Exposure of these cells to LPS induces the expression of a variety of pro-inflammatory genes that combat the bacterial infection. However, an over-reaction to LPS can lead to septic shock and death.

Many of the steps in the interaction of LPS with monocytes and endothelial cells have been elucidated in the past 10 years. The serum binding protein, LPS binding protein (LBP), delivers LPS to the phosphatidylinositol glycan-linked cell surface protein, CD14 (Figure 1B). Subsequently, the LPS-CD14 complex interacts with toll-like receptor 4 (TLR4) and its accessory protein, MD2, or is incorporated into the plasma membrane (Figure 1B). Other studies indicate that the LPS signaling complex is assembled within lipid rafts and caveolae.

LPS and oxPAPC activate some of the same intracellular signaling pathways and genes. For instance, LPS and oxPAPC also inhibit each other's induction of MCP-1 and IL-8 in endothelial cells. However, LPS and oxPAPC also exhibit differences in the pattern of gene expression. For example, E-selectin is induced only by LPS and heme oxygenase 1 is induced only by oxPAPC. A key difference between LPS and oxPAPC is that LPS is a potent activator of NF-κB, whereas oxPAPC does not activate NF-κB. Importantly, several studies have shown that oxLDL and oxidized phospholipids inhibit LPS induction of NF-κB and expression in monocytes/macrophages and endothelial cells. OxLDL inhibited LPS activation of NF-κB and induction of IL-12 and Cox-2 expression in macrophages, and lysophosphatidylcholine inhibited LPS induction of TF in monocytes. In addition, oxPAPC inhibited LPS induction of E-selectin in endothelial cells.

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However, the mechanism(s) by which oxidized phospholipids inhibited LPS induction of gene expression was not elucidated.

In this issue, Walton and coworkers report their studies of the mechanism by which oxPAPC inhibits LPS induction of IL-8 and MCP-1 in endothelial cells and macrophages. Cell fractionation and immunofluorescence were used to analyze caveolae and assembly of the LPS signaling complex (Figure 2A). They showed that oxPAPC disrupted caveolae and inhibited the assembly of the LPS signaling complex in endothelial cells. OxPAPC also inhibited TLR4- and TLR2-mediated induction of IL-8 and MCP-1 expression in human macrophages, presumably due to inhibition of the assembly of the LPS signaling complex in lipid rafts. These results suggest a common mechanism of oxPAPC inhibition of LPS signaling in endothelial cells and macrophages. OxPAPC inhibition of LPS induction of IL-8 and MCP-1 expression in endothelial cells and macrophages was selective because oxPAPC did not affect TNFα induction of these chemokines. These authors did not find evidence that oxPAPC inhibited the binding of biotinylated LPS to the cells.

In contrast to the studies by Walton and colleagues, Bochkov and coworkers proposed a different inhibitory mechanism to explain how oxPAPC inhibits LPS signaling in human endothelial cells. They showed that oxPAPC inhibited LPS signaling by blocking the binding of LPS to LBP and CD14 (Figure 2B). In addition, oxPAPC protected mice treated with a lethal dose of LPS. Binding of oxPAPC to CD14 appears to form an inactive complex because it does not engage TLR4 and activate NF-κB. The reason for the different results generated by these groups is unclear. Both studies used oxPAPC, but used different types of endothelial cells. Walton and coworkers used human aortic endothelial cells (HAECs), which express CD14 on their surfaces in a

Figure 1. Gene expression induced by mmLDL, oxLDL, and LPS. LDL can undergo mild oxidation or extensive oxidation to produce mmLDL and oxLDL, respectively. mmLDL binds to the LDL receptor as well as an undefined G protein–coupled receptor. mmLDL activates the transcription factors Egr-1, NF-AT, and PPARα and induces expression of the pro-atherogenic genes IL-8, MCP-1, and TF. OxLDL binds to the scavenger receptors SR-BI and CD36. LPS from the outer membrane of Escherichia coli binds to LBP in the serum and is delivered to CD14. The LPS signaling receptor contains TLR4 and MD2. Binding of the intracellular adaptor proteins, MyD88 and Mal, to the cytoplasmic domain of TLR4 activates NF-κB, AP-1, and Egr-1 and induces the expression of the pro-inflammatory genes, TNFα, IL-8, MCP-1, TF, VCAM-1, ICAM-1, and E-selectin.
manner similar to monocytes. Bochkov and coworkers used human umbilical vein endothelial cells (HUVECs), which utilize soluble CD14 for LPS activation. OxPAPC may bind to soluble CD14 more efficiently than membrane bound CD14, making HUVECs more susceptible than HAECs to inhibition by this pathway.

Recently, Miller and coworkers reported that mmLDL binds to CD14 via a binding site that is distinct from the LPS binding site. Furthermore, they showed that TLR4 was required for mmLDL-induced spreading of human macrophages. This suggests an additional mechanism that may contribute to the inhibitory effects of mmLDL on LPS signaling. If the TLR4/MD2 complex binds to an mmLDL-CD14 complex, it would reduce the amount of TLR4/MD2 signaling. If the TLR4/MD2 complex binds to an mmLDL-phages. This suggests an additional mechanism that may occur in atheroma of Watanabe heritable hyperlipidemic rabbits. Science. 1989;241:215–218.


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