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 oxidophospholipids, 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), was present in mmLDL and activated endothelial cells. OxPAPC also was shown to activate the transcription factors PPARγ, Egr-1, and NF-κB, and induce the expression of the pro-atherogenic genes MCP-1, IL-8, and TF in endothelial cells.

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OxPAPC both induce MCP-1 and IL-8 expression in endothelial cells and macrophages. In macrophages, and lysophosphatidylcholine inhibition of IL-12 and Cox-2 expression in macrophages, and lysophosphatidylcholine inhibited LPS induction of TF in macrophages. 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\(^\text{38}\) 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 TNF\(\alpha\) 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,\(^\text{38}\) Bochkov and coworkers\(^\text{39}\) 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.\(^\text{39}\) Binding of oxPAPC to CD14 appears to form an inactive complex because it does not engage TLR4 and activate NF-\(\kappa\)B. 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\(^\text{38}\) used human aortic endothelial cells (HAECs), which express CD14 on their surfaces in a...
manner similar to monocytes. Bochkov and coworkers\(^\text{39}\) used human umbilical vein endothelial cells (HUVECs), which utilize soluble CD14 for LPS activation.\(^\text{40}\) 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\(^\text{41}\) 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-conjugate, it would reduce the amount of TLR4/MD2 signaling. 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-conjugate, it would reduce the amount of TLR4/MD2 signaling.

In summary, two studies have reported distinct inhibitory mechanisms to explain how oxPAPC inhibits LPS signaling in human endothelial cells and macrophages. The differences may be due to the use of different cell types. It is intriguing that structural similarities between LPS and oxPAPC may permit their recognition by the pattern recognition receptor, CD14. In addition, LPS and mmLDL both bind to TLR4. LPS binding to CD14 induces an acute inflammatory response that results in septic shock. In contrast, mmLDL and oxPAPC induce chronic inflammation that contributes to atherosclerosis. Further studies are required to elucidate the role of CD14 and TLR4 in atherosclerosis. Early reports suggest a link between TLR4 and atherosclerosis\(^\text{12-24}\) consistent with the hypothesis that innate immunity may contribute to atherogenesis.\(^\text{44}\)

**References**


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