Editorial

Oxidized Phospholipid Inhibition of LPS-Signaling
A Good Side to the Bad Guys?
Clett Erridge

As chronic inflammatory processes are now understood to underpin the development of atherosclerosis, and oxidative modification of lipids and lipoproteins has long been considered to play a role in this disease, the mechanisms linking lipid peroxidation with inflammatory signaling are a key area of current atherosclerosis research. Oxidized phospholipids (OxPLs) in particular have received considerable attention in this context since their identification as key mediators of the chemokine-inducing properties of moderately oxidized low-density lipoprotein (mmLDL). OxPLs are formed not only during the oxidative modification of LDL, but also within apoptotic cell membranes, and have been shown to accumulate to reach micromolar concentrations in inflamed tissues, such as atheroma. To date, it has been widely assumed that OxPLs are predominantly proinflammatory mediators, on the basis of their ability to upregulate expression of interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 and the binding of monocytes to endothelial cells. Paradoxically, however, it has also been shown that OxPLs are potent inhibitors of inflammatory signaling induced by bacterial lipopolysaccharide (LPS, endotoxin), considered by immunologists to be a prototypic proinflammatory agent, both in vitro and in vivo.

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Bochkov and colleagues were the first to address the potential mechanisms by which OxPLs could inhibit LPS-signaling. They showed that the model OxPL oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine (OxPAPC) inhibited the binding of LPS to the serum proteins LPS-binding protein (LBP) and soluble (s)CD14, both of which serve to enhance the presentation of LPS monomers to MD2, the binding partner of Toll-like receptor (TLR)-4 that facilitates LPS-binding and recognition. Shortly afterward, Walton et al showed that OxPLs could also inhibit signaling of the related receptor TLR2, and that inhibition of LPS-signaling remained even after OxPAPC-treated cells had been washed. Walton et al therefore proposed that the target for OxPL-inhibition of LPS-signaling remained even after OxPAPC-treated cells had been washed. Walton et al therefore proposed that the target for OxPL-inhibition of LPS-signaling was instead cell-associated, and suggested that lipid rafts or caveolae could represent likely cell-bound targets, as they showed these were disrupted by OxPL treatment, potentially via OxPL-mediated increased production of ceramide. Both groups ruled out an effect of OxPLs on the intracellular signaling pathways downstream from TLR4, as it was shown that the signaling pathways induced by the tumor necrosis factor and IL-1 receptors, which are very similar to those used by TLR2 and TLR4, were not inhibited by OxPL treatment.

Thus, to date several questions have remained to be addressed regarding this aspect of OxPL function. First, as previous studies have focused largely on the model OxPL, OxPAPC, are comparable inhibitory effects observed with OxPLs derived from other classes of phospholipid? Must OxPLs remain intact to mediate their inhibition and, if so, which part of the modified molecule confers inhibitory potential? Is the target for LPS-inhibition serum-based or cell-associated? Lastly, as it has become clear that TLR-signaling potentiates atherosclerosis and the search for physiological TLR-ligands is ongoing, is it possible that OxPLs could even serve as partial agonists of TLR4, as has been proposed previously?

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, von Schlieffen and coworkers report several novel findings that help to address these outstanding questions. First, it is shown that oxidation of phospholipids with a wide variety of head groups, or sn-2 esterified unsaturated fatty acids, yields OxPLs which inhibit LPS-signaling to a similar extent, whereas unoxidized PLs are without effect. Cleavage of oxidized fatty acid residues from OxPLs is not required for inhibition, as phospholipase-resistant molecules retained activity. Together, these results suggest first that esterified and oxidized sn-2 residues appear to be the key determinant of TLR4 inhibition and second that the majority of OxPLs generated in vivo are likely to possess anti-TLR4 activity. Crucially, no evidence of partial TLR4-agonist activity of OxPLs was observed using a wide variety of cell types and markers of TLR-stimulation, whereas inhibitory activity was observed at OxPL concentrations similar to those reported to occur in inflamed tissues in vivo.

The authors also present evidence that there are multiple molecular targets for LPS-inhibition. The competitive binding of OxPLs to LBP and soluble CD14 was confirmed using elegant nondenaturing gel-based band-shift assays that revealed this binding to be noncovalent and mutually exclusive with LPS-binding. Notably, OxPLs bound rapidly and preferentially to sCD14 in both human and murine plasma, further suggesting a physiologically relevant role for OxPLs in the negative regulation of inflammation in vivo. Additionally, however, a cell-associated site of inhibition was identified, because washing of cells did not completely reverse LPS-inhibition, and the TLR4 agonist E6020, which stimulates TLR4 independently of LBP or sCD14, was also inhibited by OxPL treatment. It has been shown recently that this

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cell-associated OxPL target is likely to be MD2, the final LPS-binding protein in the presentation cascade that remains constitutively attached to and confers LPS sensitivity to TLR4.9 Thus, the “multiple-hits” of OxPL inhibition appear to be principally the extracellular proteins LBP and sCD14, and the cell-associated proteins MD2 and mCD14 (Figure). This model thus reconciles previous findings of both extracellular and cell-associated targets for inhibition,3,4 and is equivalent to that established for existing LPS-antagonists, such as lipid-IVa and the drug Eritoran. The recent observation that OxPLs do not inhibit signaling via TLRs that function independently of MD2, LBP, and CD14, further supports this model, and further suggests that disruption of lipid-rafts by OxPLs is not responsible for inhibition of TLR-signaling.9

Taken together, these findings, while answering many previous questions regarding OxPL function, leave several larger questions to be addressed. For example, if OxPLs are not partial TLR-agonists, then what else may constitute physiological ligands for TLRs during atherosclerosis? Moreover, these findings add further fuel to the debate as to whether OxPLs are predominantly proinflammatory or antiinflammatory in nature. Certainly, OxPLs promote expression of IL-8 and MCP-1 and the binding of monocytes to endothelial cells. However, as shown in the present and previous studies,3,8,9 OxPLs do not induce expression of many classical markers of inflammation, such as TNF-α, IL-1β, ICAM-1, and VCAM-1, which are established to promote atherosclerosis, whereas they do upregulate antiinflammatory genes such as MAP-kinase phosphatases and haem-oxygenase-1 and potently inhibit TLR2 and TLR4 signaling. Given this apparent duality of function, future studies will be required to investigate whether OxPLs serve more as the good guys or as the bad guys in inflammatory diseases.

Disclosures

None.

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