HHS Public Access

Author manuscript

Free Radic Biol Med. Author manuscript; available in PMC 2018 October 01.

Published in final edited form as:

Free Radic Biol Med. 2017 October ; 111: 156–168. doi:10.1016/j.freeradbiomed.2017.02.035.

The effect of oxidized phospholipids on phenotypic polarization and function of macrophages

Vlad Serbulea, **Dory DeWeese**, and **Norbert Leitinger, PhD**

Robert M. Berne Cardiovascular Research Center and Department of Pharmacology, University of Virginia

Abstract

Oxidized phospholipids are products of lipid oxidation that are found on oxidized low-density lipoproteins and apoptotic cell membranes. These biologically active lipids were shown to affect a variety of cell types and attributed pro-as well as anti-inflammatory effects. In particular, macrophages exposed to oxidized phospholipids drastically change their gene expression pattern and function. These 'Mox,'macrophages were identified in atherosclerotic lesions, however, it remains unclear how lipid oxidation products are sensed by macrophages and how they influence their biological function. Here, we review recent developments in the field that provide insight into the structure, recognition, and downstream signaling of oxidized phospholipids in macrophages.

Keywords

Oxidized phospholipids; macrophages; inflammation

Introduction

Since the discovery of lipid oxidation products as biologically active compounds by Hermann Esterbauer[1–3], the presence of oxidized lipids has been confirmed in a variety of tissues that are either chronically inflamed or oxidatively damaged. Lipid oxidation is associated with the pathogenesis of various diseases including atherosclerosis, diabetes, cancer, Alzheimer's disease, rheumatoid arthritis, and the pathophysiology of aging. In all of these settings, macrophages play a key role in controlling the initiation and progression of the disease. Phospholipids that contain unsaturated fatty acids are major constituents of cell membranes and lipoproteins and are particularly prone to oxidative modification, which results in the formation of a large variety of biologically active compounds. Oxidized phospholipids (OxPL) derived from oxidation of lipoproteins or from apoptotic cell membranes were shown to accumulate at sites of chronic inflammation and oxidative tissue damage, where they not only affect biophysical properties of membranes, but also can be considered endogenous danger associated molecular patterns (DAMPs) that modulate immune responses (reviewed in [4–7]). After the identification of OxPL as biologically

active components of minimally oxidized LDL[8, 9], OxPL have been ascribed pro- as well as anti-inflammatory properties, depending on their structural features and functional groups, which also determine their recognition by extracellular or intracellular receptors. The effects of OxPL on modulation of inflammatory responses have been extensively reviewed[10–14], however, little is known about the mechanisms that control the formation and degradation of OxPL in different cell types. Moreover, the effects oxidized lipids have on cells of the immune system are poorly understood. OxPL are recognized by macrophages via various mechanisms involving intra- and extracellular receptors, eliciting a series of responses that result in context-specific phenotypic adaptation. Here, we will summarize recent findings that illustrate how macrophages and other cells recognize and respond to OxPL and we will discuss the functional consequences of these responses for inflammatory control and tissue homeostasis.

Structures and biological functions of oxidized phospholipids

Oxidative modification of lipids is mediated by free radical-induced mechanisms involving enzymes such as NADPH oxidase and myeloperoxidase. Thus, at sites of inflammation, where neutrophils and other cells generate an environment of high oxidative stress, lipid oxidation products accumulate and exert a variety of biological activities. Polyunsaturated fatty acids and especially arachidonic acid are highly susceptible to lipid peroxidation, which leads to the generation of lipid hydroperoxides, which can undergo carbon-carbon bond cleavage giving rise to the formation of short chain, unesterified aldehydes and aldehydes still esterified to the parent lipid, termed core-aldehydes. It has been shown that activation of the NADPH oxidase during apoptosis leads to oxidation of the membrane phosphatidylserine (PS), but also phosphatidylcholine (PC) and phosphatidylethanolamine (PE)[15]. The presence of oxidized PC (OxPC) on the surface of apoptotic cells has been demonstrated using the monoclonal antibody EO6, which exclusively binds to OxPC[16–19]. Furthermore, enzymatic oxidation of phospholipids involving 12/15 lipoxygenase produces biologically active mediators[20, 21]. The cholesteryl esters found within LDL are also subject to oxidative modification, which may contribute to endothelial activation[22]. Miller et al. found that oxidized cholesteryl esters (OxCE) use toll-like receptor 4 (TLR4) and spleen tyrosine kinase (Syk) to induce a pro-inflammatory response in macrophages[23].

Initially, three biologically active phospholipids present in minimally modified low-density lipoprotein (mm-LDL), which were derived from oxidation of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC), were structurally identified: 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphorylcholine (POVPC) and 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine (PGPC), and 1-palmitoyl-2-(5,6-epoxyisoprostane)-PC (PEIPC)[8, 24]. These lipids were shown to activate endothelial cells in a structure-specific manner[25, 26]. Furthermore, a group of CD36-activating truncated oxidized phospholipids was described by Podrez et al. These species include an *sn*-2 acyl group that requires a γ-hydroxy-α,β-unsaturated carbonyl or a γ-oxo-α,β-unsaturated carbonyl and were found to be generated during LDL oxidation[27]. These lipids, collectively referred to as $oxPC_{CD36}$, were found to directly contribute to the development of foam cell formation in macrophages[28]. In this study the authors describe that even trace amounts of $oxPC_{CD36}$

are enough to induce CD36-dependent binding and uptake of LDL[28]. Accumulating evidence suggests that biologically active OxPL that are present in atherogenic lipoproteins, in atherosclerotic lesions, and in membrane vesicles released from activated and apoptotic cells play an essential role in the development of atherosclerosis [14, 29, 30]. Indeed, OxPL affect a variety of vascular cell types, including endothelial [31–36] and smooth muscle [37–39] cells. Furthermore, OxPL have potent effects on immune cells. Studies performed by our lab have shown that phospholipid oxidation products of PAPC (OxPAPC) inhibit basic steps of the classical dendritic cell (DC) maturation process[7, 40]. Inhibitory effects on DC, anergic effects on T-cells as well as on the formation on T_{reg} cells have been reported[41, 42], and one report demonstrates an important role for OxPL on epigenetic modulation of DC activation[41].

Levuglandins/ketoaldehydes are derived from oxidation of arachidonic acid and contain highly reactive aldehydes, which readily form lysyl adducts with proteins[43]. Macrophages recognize LDL that has been modified with levuglandins[44], and free levuglandins as well as protein-adducts have been demonstrated at sites of inflammation and oxidative stress, including atherosclerotic lesions and kidneys of patients with end-stage renal disease[45, 46]. However, the exact role of levuglandins/ketoaldehydes in contribution to disease progression remains to be elucidated. Given the ability of levuglandins/ketoaldehydes to react with primary amines, it was postulated that levuglandins might also react with phosphatidylethanolamines. Indeed, it was shown that γ-ketoaldehydes are able to react with ethanolamine at a much faster rate than with lysine[47, 48] to produce compounds that induce inflammatory reactions in macrophages[49]. The γ-ketoaldehyde/levuglandin modification of phosphatidylethanolamines produces a highly cytotoxic phosphatidylethanolamine[50], and using mouse models of hypertension, it was shown that scavenging γ-ketoaldehydes prevented immune-mediated hypertension and associated comorbidities[51]. Another modification of phosphatidylethanolamines has been described to result in the formation N-acyl-phosphatidylethanolamines (NAPEs), which are precursors to N-acyl-ethanolamines (NAEs), thought to be highly potent satiety signals generated in the intestines[52]. Recently, a derivative of carboxyalkylpyrrole(CAP)-phosphatidylethanolamine (CAP-PE) has been identified in the plasma of ApoE−/− mice[53]. These CAP-PE derivatives directly bind and activate TLR2/1 to induce platelet activation[53]. Similarly to the phosphatidylethanolamine adducts, the CAP-protein derivatives, such as those formed by ω-(2-carboxyethyl)-pyrrole (CEP), have also been shown to induce platelet activation, but even more startlingly, they induce VEGF receptor-independent angiogenesis through TLR2 activation[54]. Moreover, the CEPs have been extensively described for their role as inducers of inflammation in age-related macular degeneration[55].

OxPL and macrophages: Identification of Mox

Macrophages sense changes in their tissue microenvironment and respond by changing their phenotype and function accordingly. In chronically inflamed tissues, the formation of OxPL may contribute to the initiation of the macrophage-dependent inflammatory process, but also to the perpetuation of inflammation, possibly by interfering with inflammatory resolution. Several studies have reported a variety of cellular responses to an oxidatively damaged

microenvironment by analyzing the reaction of macrophages to OxPL[56–59]. Of special interest, it has been noted that the profiles of inflammatory gene expression and recruitment of monocytes and macrophages induced by oxidized lipids is remarkably similar to the "low-grade inflammation" seen in settings of diet-induced obesity or atherosclerosis, and generally in metabolic tissue damage[60, 61]. Furthermore, we have shown that OxPL induce specific macrophage recruitment in the air-pouch model of inflammation, by a mechanism involving CCR2[62].

A critical event leading to the resolution of inflammation is the removal of apoptotic cells[63, 64]. Delayed apoptosis results in increased oxidative stress and diminished phagocytosis, and may be associated with the prolongation and persistence of inflammatory disorders[65, 66]. These observations strongly indicate that improved tissue remodelling could be a direct consequence of increased engulfment capacity of macrophages. Taking into consideration that apoptosis is essential for successful resolution of acute inflammation, OxPL generated during apoptosis could influence this process, by upsetting the balance of macrophage phenotypes. In this context, OxPL were shown to be important recognition signals on apoptotic cells[18], which could facilitate phagocytosis by macrophages[15, 67]. The Tabas group showed that OxPL induce apoptosis in macrophages undergoing ER-stress via CD36/TLR2 activation[68] and Gerhard Kronke's group reported that 12/15 lipoxygenase plays a major and unexpected role in the non-inflammatory clearance of apoptotic cells, via a mechanism involving the formation of oxidized phosphatidylethanolamines (OxPE)[20]. On the other hand, phagocytosis was shown to be inhibited in macrophages by mm-LDL[69] and OxPAPC[58], and Sylvia Knapp's group discovered that this inhibition involves WAVE1[70, 71], which is a cytoskeleton bound A-kinase anchoring protein. The group showed that WAVE1 knockout macrophages are resistant to OxPAPC-inhibited phagocytosis, and that the E. coli infected chimeric WAVE1-knockout mice have a better survival rate compared to controls[71]. Altogether, this has important implications in the regulation and outcome of inflammatory responses in atherosclerosis and sepsis.

In chronically inflamed tissues, where macrophages are constantly exposed to high levels of oxidized lipids, macrophages survive for surprisingly long periods of time, despite the toxic environment[72]. This implies the upregulation of survival mechanisms, such as antioxidant pathways and phase II detoxification genes. We have previously shown that UV-light-induced oxidation of phospholipids have protective effects on skin cells, via induction of redox-sensitive transcriptional programs involving activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2)[73, 74]. In atherosclerosis, the intra-plaque milieu is complex, leading to a remarkably diverse macrophage population[75]. Within an atherosclerotic plaque, lipid mediators ranging from cholesterol crystals to OxPL activate the inflammasome and pattern recognition receptors, as well as other receptors to induce both pro- and anti-inflammatory phenotypic polarizations (M1 and M2)[76]. Our group characterized a novel OxPL-induced macrophage phenotype, "Mox", in murine atherosclerotic lesions[58]. The Mox phenotype significantly differs from pro-inflammatory "M1" and anti-inflammatory "M2" macrophages and is characterized by an Nrf2-dependent gene expression pattern[58]. Nrf2-dependent gene expression is induced by OxPL in the vasculature and especially in endothelial cells[77, 78] where it is believed to exert protective

effects. However, it was also shown that Nrf2 deficiency in macrophages had differing effects on atherosclerotic lesion formation[79–81] and we have shown that Nrf2 deficiency in macrophages did not protect against HFD-induced insulin resistance[82]. Nrf2 in macrophages was shown to exert direct anti-inflammatory effects[83] and an anti-inflammatory, reparative function in the brain was ascribed to Mox macrophages detected in microglia after stimulation of nicotinic receptors[84]. Of note, a recent report demonstrated regulation of chronic inflammatory pain by OxPAPC in neurons via activation of TRPA1 channels[85].

Interestingly, based on their specific gene expression profile, Mox macrophages have been described to play a role in iron metabolism, favouring iron storage due to compromised iron release[86]. These and other observations also indicate that Mox contribute to inflammation. We have shown that OxPL induce a low level of inflammatory gene expression through a TLR2-dependent mechanism[87]. Moreover, we were able to show that Mox macrophages secrete IL-1β[58]. In macrophages, the events leading up to IL-1β secretion involves the transcription of pro-IL-1β, activation of the NLRP3 inflammasome, which constitutes an activated caspase-1, which then cleaves pro-IL-1β. However, the endogenously formed activators of IL-1β production in chronically inflamed tissue are not known. In settings of ‘sterile inflammation,’ such as in obesity and atherosclerosis, the underlying question whether products of lipid oxidation could evoke an inflammatory response via inflammasome-dependent cytokine production remains unanswered. In this regard, OxPL were shown to induce NLRP3 inflammasome activation in macrophages[88] and Jonathan Kagan’s group recently showed that endogenously formed OxPAPC is able to directly activate caspase 11, thereby activating the NLRP3 inflammasome in a non-canonical fashion in dendritic cells[89]. These findings could have important implications for a variety of diseases where caspase 11 activation and inflammasome-dependent activation of the immune response has been reported[90].

The cellular metabolic programming, or bioenergetics, of macrophages has been linked to a macrophage’s inflammatory phenotype: pro-inflammatory “M1” macrophages rely upon glycolysis for their bioenergetic needs while anti-inflammatory “M2” macrophages rely on oxidative phosphorylation[91–95]. Recently, multiple studies identified the importance of cellular metabolism in the determination of macrophage pro-[96] or anti-inflammatory[97] function. Further investigation revealed that the mitochondrial dysfunction caused by pro-inflammatory stimuli prevented macrophages from assuming an anti-inflammatory polarization state[98].

However, the metabolic profile of Mox has not been examined. Furthermore, the mechanisms by which OxPL induce inflammatory signaling and metabolic changes in Mox remain poorly defined. OxPL can induce ceramide accumulation in macrophages[99], and since mitochondrial damage can be mediated by ceramides, Mox macrophages may have a compromised bioenergetic profile. Activation of TLR2 signaling by OxPL may contribute to the low-grade chronic inflammation, and ceramide synthesis was shown to be induced by TLR2 stimulation[100]. TLR2 is known to hetero-dimerize with either TLR1 or TLR6, which collaborate with OxPL receptors CD14 or CD36 respectively[68, 101, 102]. Together,

these studies indicate that OxPL may affect macrophage bioenergetics via mechanisms involving TLR2-dependent ceramide production.

How are OxPL recognized?

Among a number of recently characterized danger signals, phospholipid oxidation products accumulate under conditions of increased oxidative stress and cell death. They serve as indicators of inflammation-induced tissue damage and were shown to act as endogenous regulators of the innate immune response[4]. For instance, diet-induced oxidative stress is a major cause for vascular, liver and adipose tissue damage, hallmarks of atherosclerosis, fatty liver disease and insulin resistance and diabetes[103]. Disturbed redox balance due to chronic inflammation in neurological disorders may contribute to formation and accumulation of OxPL in the brain[104]. Nevertheless, how the recognition of an altered tissue microenvironment by immune cells and the mechanism by which oxidative damage ultimately translates into an inflammatory reaction is not clear.

Based on the current literature, OxPL may bind to and initiate a response through soluble acceptors in the blood plasma, by membrane-bound receptors, or by intracellular sensor proteins. Depending on their functional reactive groups, they are able to either covalently modify these receptor proteins, or possibly bind to and interact in a reversible manner. In inflamed tissue, OxPL can either act pro-inflammatory or anti-inflammatory, and for most OxPL whose biological functions have been investigated, dual pro and anti-inflammatory effects were described. So far, OxPL have been documented to directly bind to LPS binding protein (LBP), CD14, MD-2, and CD36, which may lead to activation or inhibition of TLR4 or TLR2 signaling. Intracellularly, OxPL interact with caspase 11 or Keap1/Nrf2, exerting pro- or anti-inflammatory effects, respectively. Here we summarize the evidence for the various systems that recognize and respond to OxPL. Table 1 catalogs the evidence for OxPL effects as distinguished by phospholipid class (i.e. PC, PE, PS, PG, PA, and PI). It is important to keep in mind that many studies were performed with mixtures, which contain a large number of diverse oxidized moieties, making it difficult to conclude which oxidized moieties are tied to which downstream consequences. Nonetheless, the studies that include enriched, purified, or synthetic OxPL species are included in Table 2.

OxPL modify proteins at the cell membrane and in the cytosol

Using cultured RAW 264.7 macrophages and fluorescently labeled POVPC or PGPC Hermetter's group identified primary protein targets of these phospholipids by mass spectrometry[105]. They showed that the aldehyde-containing POVPC binds to proteins in the cell membrane by covalently reacting with amino groups of proteins. Interestingly, POVPC was exchangeable from lipoproteins to cells, where only a selective group of proteins was modified by labeled POVPC. PGPC on the other hand, freely travelled through the cell membrane into the cytosol[105]. Berliner's group took a different approach to identify the proteome that is modified by OxPL in endothelial cells, using biotin as affinity tag at the polar head group of the phospholipids[106]. They then showed that the interaction of OxPL with proteins involves modification of cysteines[107].

OxPL are DAMPS that are recognized by pattern recognition receptors

Upon oxidative modification, phospholipids structurally resemble DAMPs that are recognized by pattern recognition receptors (PRRs)[5]. Sensing of these endogenously formed danger or “altered-self” molecules by the innate immune system is mediated by immune-modulating and scavenger receptors such as CD36, TLRs, CD14, LBP, and C-reactive protein[108, 109]. The fact that the oxidation process renders phospholipids “visible” to the innate immune system indicates a crucial role for phospholipid oxidation products in the pathogenesis of both chronic inflammatory and autoimmune diseases[4].

First indications that OxPL bear similarities with compounds that can be recognized by TLRs came from our studies showing that these lipids potently inhibit bacterial ligand-induced TLR4 activation[102]. Our group was first to show that OxPAPC inhibited LPS-induced NF-κB-mediated inflammatory gene expression in human umbilical-vein endothelial cells (HUVEC) and in LPS-injected mice protecting mice from lethal endotoxin shock[102]. We further demonstrated that OxPAPC bound to LPS-binding protein (LBP) and CD14, which blocked the interaction of LPS with both proteins *in vitro*[102]. These results were supported by others[101, 110–112], showing that OxPAPC can bind to CD14 and MD2 to interfere with LPS-TLR4/MD2 activation. Moreover, OxPAPC inhibited N-palmitoyl-S-dipalmitoylglyceryl-Cys-Scr-$(lys)_4$ (Pam_3CSK_4)-induced TLR2 activation, which was reversed by serum and sCD14, suggesting that CD14 is one of the targets of OxPAPC.

Oxidative modification of phospholipids provides epitopes for the adaptive immune system

The patterns that are generated during oxidation of phospholipids are also recognized by the humoral part of the adaptive immune system. Both IgG and IgM antibodies directed against oxidized low-density lipoprotein (oxLDL) are present in the plasma of humans and animals and their titers have been shown to correlate with atherosclerosis progression and measures of lipid peroxidation[113, 114], as well as in several autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis[115]. Detailed studies in ApoE-deficient mice, which show increased levels of oxLDL, led to the cloning of a set of abundant monoclonal IgM antibodies directed against oxLDL, which includes the prototypic EO6 antibody that specifically binds to OxPL on the surface of oxLDL and apoptotic cells[17, 116, 117]. This antibody recognizes the PC headgroup, which is exposed after conformational changes following the oxidation of the phospholipid[118].

OxPL bind to soluble immune receptors

CD14—CD14 is a co-receptor for both TLR2 and TLR4 that has been shown to exist in both a membrane-bound and soluble form. CD14 has been shown to enhance the sensitivity of TLR2 activation to Pam_3CSK_4, and is essential for LPS-induced activation of TLR4. As shown by Erridge et al., OxPAPC, in particular the components PGPC and POVPC, are able to potently inhibit Pam_3CSK_4 activation of TLR2, in a HEK cell model with TLR2-overexpression[111]. The authors concluded that this inhibition was likely due to the binding of OxPL directly to CD14, due to the rescue of the inhibition upon supplementation with soluble CD14. Furthermore, Valery Bochkov’s group found that OxPL mixtures with the

head groups PE, PS, and PA induced a mobility shift in soluble CD14, indicating a direct binding interaction of these phospholipids to CD14[101]. OxPL with the PC head group also bind to CD14, but do not cause a mobility shift due to the overall neutral charge of the complex. The structural requirements for OxPL binding to CD14 require further investigation, so far there is experimental evidence for PECPC, PEIPC, OxPAPE, OxPAPC, and OxPAPS to interact with CD14[101, 111, 119] (Table 2). Walton et al. ruled out an involvement of CD14 in transmitting pro-inflammatory effects of OxPAPC on endothelial cells, instead identifying another GPI-anchored protein recognizing OxPL[120].

LPS binding protein—OxPAPC and a number of purified components of OxPAPC are able to directly bind to LBP and inhibit LPS action[101, 102, 111, 112, 121]. Unlike for CD14, it was found that oxidized phospholipids with any head group (PC, PE, PS, and PA) were able to inhibit the binding of LPS to LBP (Table 1). Eighteen total OxPL have been documented to interact with LBP, out of the twenty-four investigated. This implies that there is little chemical specificity in the interaction, and that for the most part, oxidation of the phospholipid is the only prerequisite for inhibitory interaction with LBP. It has never been determined whether LBP is required for OxPL-induced inflammation.

MD2—Another study found that the oxidized phospholipid KOdiA-PC directly inhibits the binding of LPS to MD2, providing yet another mechanism by which OxPL are able to inhibit LPS action[122]. This was supported by other studies using both binding experiments and functional assays to indicate that OxPAPC can compete with LPS to bind MD2[111]. The structural requirements of OxPL needed to activate or inhibit MD2-dependent signaling remain unknown. Taken together, these findings indicate that OxPL can compete with serum-soluble factors to inhibit LPS, and likely other bacterial or viral components, which induce systemic inflammation. This mechanism of scavenging accessory proteins by OxPAPC, or by inhibiting TLR-signaling may represent a negative feedback during inflammation to blunt innate immune responses and provide protection from overshooting inflammatory reactions.

Membrane receptors recognizing OxPL

CD36—CD36 is membrane protein known to have properties of fatty acid binding and uptake, and is expressed on a wide variety of cells, including macrophages. Podrez et al. classified a series of short-chain OxPCs, which bind to and activate CD36 on macrophages and platelets[27, 123]. They also found that adding these OxPL to cholesterol-containing liposomes enhanced their CD36-mediated uptake into macrophages[28]. The Hazen group further identified OxPS on apoptotic cells as an essential component for CD36-mediated macrophage phagocytosis of apoptotic cells[67]. Within this same study, it was shown that loading cells with oxidized phosphatidylserines, but not non-oxidized PS, facilitated their uptake by macrophages. There is evidence for CD36 activation by the following species of OxPL: OxPAPC, LysoPC, PGPC, POVPC, HOOA-PC, KOOA-PC, HOdiA-PC, and KOdiA-PC (Table 2). As mentioned previously, it was also found that the same individual species with PS head-group also bind CD36.

TLR2—Our lab has shown that OxPAPC induces pro-inflammatory gene expression in macrophages via a mechanism that involves TLR2[87] . TLR2 dimerizes with other TLRs, namely TLR1 and TLR6, to recognize distinct sets of ligands[124]. TLR2 heterodimers interact with CD36 or CD14, so it is possible that OxPL induce TLR2-dependent signaling via binding to these accessory receptors. The crystal structure of TLR2 with TLR1 and ligand Pam_3CSK_4 was elucidated in 2007 and with it, a better understanding of the TLR2 binding pocket[125]. Compared to conventional TLR2 agonists, such as lipoteichoic acid (LTA) or Pam_3CSK_4, OxPL have been shown to function as weak agonists for TLR2, with agonistic activity mainly residing in the long-chain fraction of OxPAPC[87]. Previously, only LysoPC[126] and LysoPS[127] purified species have been shown to *activate* TLR2. Based on these studies, the activation of TLR2 is not head group specific, as noted by the inclusion of both PC and PS head groups in the TLR2-activating lipids. As of now, there is no published evidence to support whether PE species activate TLR2. OxPL species that have been described to *inhibit* the action of TLR2 include PGPC, POVPC, PECPC, PEIPC, KOdia-PC, OxPAPC and OxPAPE[40, 110, 111].

TLR2-deficiency in mice was shown to be protective in various settings of non-infectious inflammatory disease models including atherosclerosis, reperfusion injury, and diabetes[128–130]. Oxidative tissue damage is a prominent feature in these diseases, and as such, TLR2 recognition of oxidatively modified DAMPs may influence disease initiation and progression. However, further studies are necessary to fully understand the structural requirements of OxPL for either activation or inhibition of TLR2.

TLR4—While there are many reports demonstrating that oxidized phospholipids inhibit TLR4-mediated effects[40, 101, 102, 110, 111], there is controversial evidence to date to support a role for TLR4 in OxPL-induced inflammation. While some studies imply TLR4 in mediating effects of OxPL[131] or LysoPC[126], others have ruled out an involvement of TLR4 in OxPL-mediated pro-inflammatory effects[132]. OxPL species that have been shown to *inhibit* LPS-induced TLR4 activation include PGPC, POVPC, PECPC, PEIPC, OxPAPC and OxPAPE (Table 2).

TLR9—A recent report showed TLR9 to be activated by CEP in platelets, thereby increasing platelet reactivity[133]. These findings further imply an important role for lipid oxidation in the control of thrombogenicity[53, 123, 134, 135], and increase the spectrum of immune receptors recognizing endogenously formed oxidation products.

GPCRs—A number of GPCRs have been implicated to play a role in recognizing and transmitting signals induced by OxPL. Due to structural similarities of fragmented OxPL with platelet activating factor (PAF) and the finding that PAF-receptor antagonists could inhibit some effects of OxPL[136, 137], the PAF-receptor has been implicated as one of the major receptors[138–140]. Other GPCRs that have been shown to be involved in mediating effects of OxPL include the prostaglandin E2 (EP2) receptor[141], the S1P1 receptor[142], VEGF receptor[143], and GRP-78[144].

Intracellular ‘receptors’ that sense OxPL—Intracellular sensors for OxPL exist, however, whether sufficient amounts of OxPL are produced inside the cell or if they have to

be transported across cell membranes has not been elucidated. However, it has been shown that some OxPL species, including PGPC, readily cross the cell membrane[105] and Tom McIntyre's group identified TMEM30a as a transport channel, facilitating OxPL traffic from outside the cell to the mitochondria[145].

Keap1/Nrf2—Nrf2 is a cytosolic redox-regulated transcription factor that is bound by Keap1, a redox-sensitive chaperone protein that promotes the degradation of Nrf2. Upon oxidative or electrophilic stress, Keap1 is thought to undergo a conformational change, disrupting its ability to bind Nrf2, allowing Nrf2 to translocate to the nucleus and promote transcription of antioxidant response systems[146]. The mechanism by which OxPL activate Nrf2-dependent gene expression may be by direct binding of electrophilic functional groups (such as those containing cyclopentenones) to Keap-1. On the other hand, OxPL have been shown to activate protein kinase C (PKC)-dependent signaling[10], and Nrf2 was shown to be phosphorylated at Ser-40 by PKC[147, 148].

Caspase 11/NLRP3—Recently a study showed the ability of OxPAPC to directly activate caspase11 and subsequently the NLRP3 inflammasome in dendritic cells[89]. These authors demonstrated direct binding of certain species of OxPAPC, likely PEIPC, to caspase11 in a similar manner as intracellular LPS would bind to and activate caspase 11. Others have shown that OxPL induce NLRP3 activation in macrophages[88].

PPARγ—Among nuclear hormone receptors, peroxisome proliferator-activated receptor-γ (PPARγ) was shown to recognize OxPL. McIntyre's group showed that OxPL activate PPARγ-dependent gene expression, leading to the expression of cyclooxygenase 2 in monocytes[149, 150].

Conclusion and Outlook

Together, oxidative modification of phospholipids represents a common underlying mechanism in many diseases where tissue damage is involved. The formation of OxPL seems to be a general feature in chronic inflammatory settings that often lead to debilitating states in many patients. Therefore, attempts to lower OxPL levels are promising approaches to combat inflammation and tissue damage[151]. Not surprisingly, OxPL can be used as predictive biomarkers for certain diseases[152]. On the other hand, some commercial enterprises have explored the use of Lecinoxoids, which are 'oxidized phospholipid-like small molecules,' for anti-inflammatory therapies[153–155]. For instance, Lecinoxoid VB-201 had a notable effect in a mouse model of experimental autoimmune encephalomyelitis[155], and inhibited CD14 and TLR2-dependent inflammation[154]. Devising novel strategies for lowering OxPL levels as well as exploiting anti-inflammatory properties by developing small molecules modeled after OxPL should produce novel therapies against chronic inflammatory diseases.

Acknowledgments

This work has been supported by NIH grant R01 DK096076 to N.L.; V.S. is a recipient of a SFRBM Mini-Fellowship and is supported by an NIH F31 pre-doctoral fellowship. D.D and V.S. are also supported by a Double-

Hoo grant from the University of Virginia. The authors apologize to the many authors whose excellent work could not be cited due to space limitations.

Abbreviations

CAP	carboxyalkylpyrrole
CAP-PE	carboxyalkylpyrrole-phosphatidylethanolamine
CEP	carboxyethyl-pyrrole
DAMPs	danger associated molecular patterns
DC	dendritic cell
HUVEC	human umbilical-vein endothelial cells
LBP	LPS binding protein
LTA	lipoteichoic acid
mm-LDL	minimally modified low-density lipoprotein
NAEs	N-acyl-ethanolamides
NAPEs	N-acyl-phosphatidylethanolamines
Nrf2	nuclear factor (erythroid-derived 2)-like 2
OxCE	oxidized cholesteryl esters
oxLDL	oxidized low-density lipoprotein
OxPAPC	oxidation products of PAPC
OxPC	oxidized phosphatidylcholine
OxPL	oxidized phospholipids
PAF	platelet activating factor
Pam_3CSK_4	N-palmitoyl-S-dipalmitoylglyceryl-Cys-Scr-$(lys)_4$
PAPC	1-palimitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PEIPC	1-palmityoyl-2-(5,6-epoxyisoprostane)-sn-glycero-3-phosphorylcholine
PGPC	1-palmityoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine
PKC	protein kinase C
POVPC	1-palmityoyl-2-oxovaleroyl-sn-glycero-3-phosphorylcholine

PPARγ	peroxisome proliferator-activated receptorγ
PRR	pattern recognition receptors
PS	phosphatidylserine
Syk	spleen tyrosine kinase
TLR4	toll-like receptor 4

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Highlights

- Oxidized phospholipids (OxPL) are endogenous danger-associated molecular patterns.
- Receptors that interact with OxPL include CD14, TLR2, CD36, Nrf2, and Caspase 11.
- OxPL induce a pro-inflammatory response in macrophages.
- OxPL antagonize the effects of pathogen-associated molecular patterns.
- We provide a summary of structure-function relationships of diverse OxPL species.

Table 1

Evidence for oxidized phospholipid recognition sorted by head group

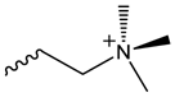
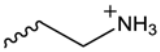
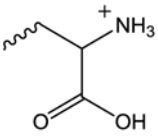
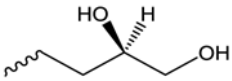
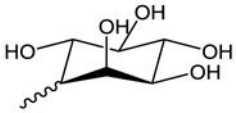
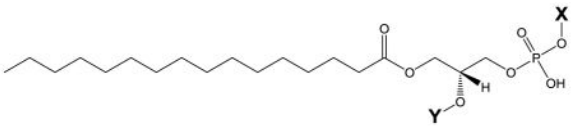
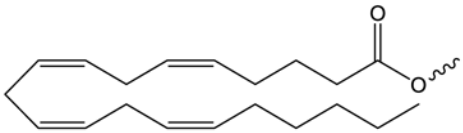
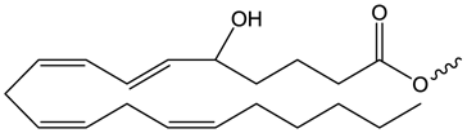
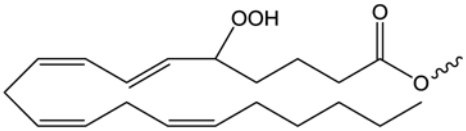
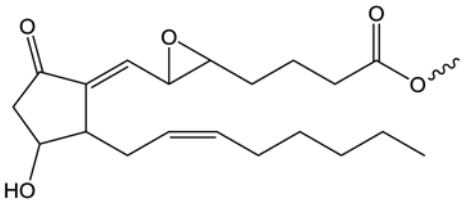
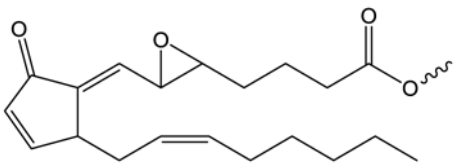
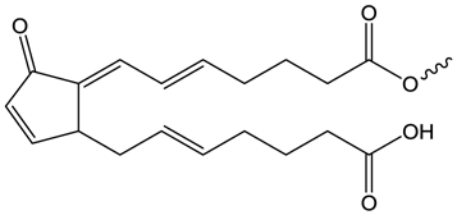
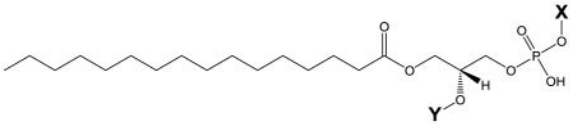
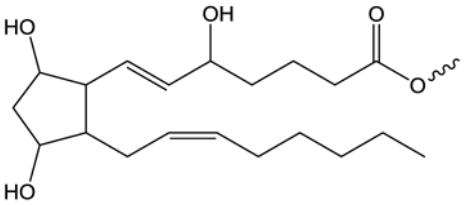
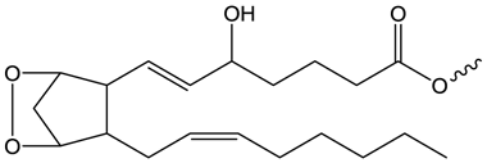
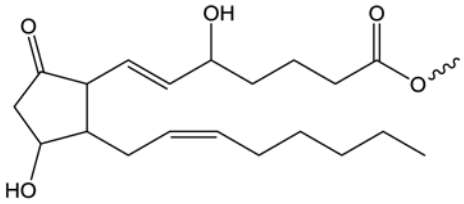
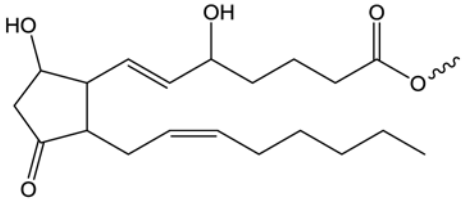
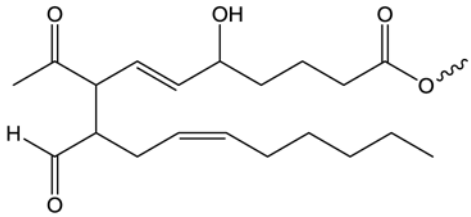
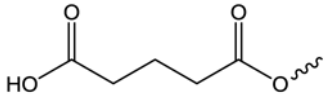
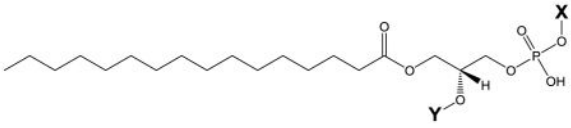
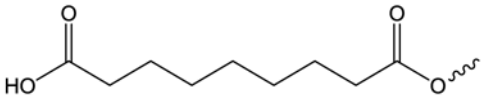
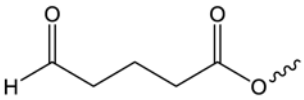
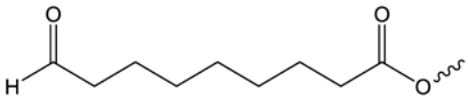
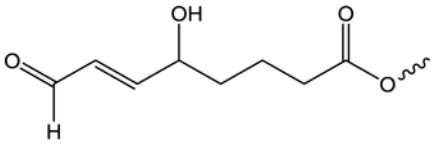
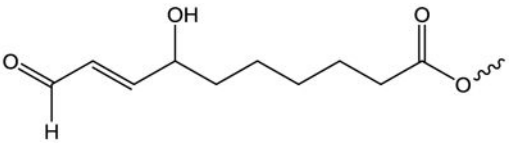
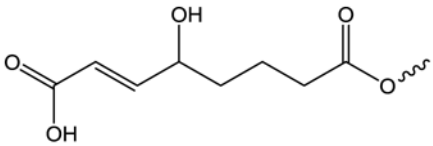
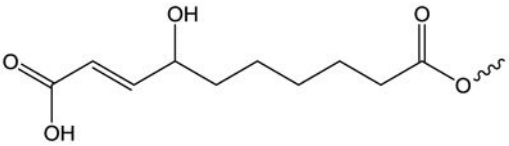
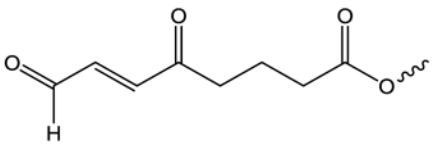
Oxidized Phospholipid Class	Head Group	Structure (X)	Activation	Inhibition
OxPC	Choline		CD36[120], Nrf2[77, 78], Casp11/NLRP3[89], TLR2[87]	TLR2[40, 111], TLR4[111, 112], CD14/MD2[111], LBP[101]
OxPE	Ethanolamine		CD36[67, 78, 118], Nrf2[78]	TLR2[111], TLR4[110, 111], CD14[101], LBP[101]
OxPS	Serine		CD36[67, 78], Nrf2[78]	CD14[101], LBP[101]
OxPA	Phosphatidic Acid		Nrf2[78]	LBP[101]
OxPG	Glycerol		Nrf2[78]	N/A
OxPI	Inositol		N/A	N/A

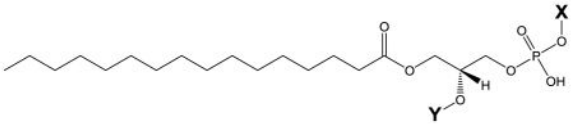
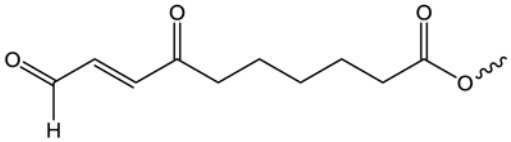
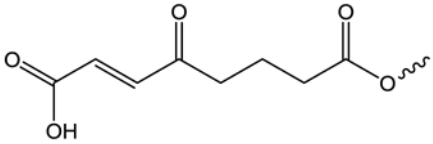
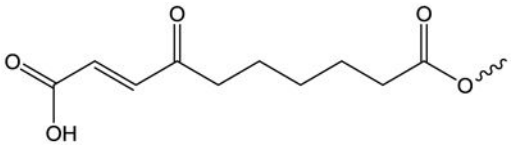
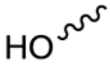
Table 2

Catalog of oxidized phospholipids by epitope

General Phospholipid Structure			
***sn*-2 Moiety**	**Structure (Y)**	**Abbreviation (C16:0 in *sn*-1)**	**Associated Head Group (X)**
Arachidonoyl		PA-	PC, PE, PS, PA, PG, PI
Oxygenation			
Hydroxy- arachidonoyl		PA(X)-OH or HETE-(X)	PC[78, 156–158], PE[156–158], PS
Hydroperoxy- arachidonoyl		PA(X)-OOH or HPETE-(X)	PC[78], PE, PS
5,6-Epoxyisoprostane E2		PEI-(X)	PC[24, 78, 89]
5,6- Epoxycyclopentenone		PEC-(X)	PC[119, 159]
15-deoxy-Δ12,14- Prostaglandin J2		15d-PGJ2-(X)	PC[119, 160]

General Phospholipid Structure			
***sn*-2 Moiety**	**Structure (Y)**	**Abbreviation (C16:0 in *sn*-1)**	**Associated Head Group (X)**
F2-Isoprostane		F2-IP-(X)	PC[161]
H2-Endoperoxide Isoprostane		H2-IP-(X)	N/A
E2-Isoprostane		E2-IP-(X)	N/A
D2-Isoprostane		D2-IP-(X)	N/A
E2-Isolevuglandin		E2-IL-(X)	PC[162, 163]
Chain Fragmentation			
Glutaroyl		PG-(X)	PC[8, 78] [25, 26, 76, 136, 164], PE, PS

General Phospholipid Structure			
***sn*-2 Moiety**	**Structure (Y)**	**Abbreviation (C16:0 in *sn*-1)**	**Associated Head Group (X)**
Azelaoyl		PAze-(X) or PAz- (X)	PC[78, 165, 166], PE, PS
5-Oxovaleroyl		POV-(X)	PC[8, 26, 78, 164], PE, PS
9-Oxononanoyl		PON-(X)	PC[16, 164], PE, PS
5-Hydroxy-8-oxo-6- octenoyl		HOOA-(X)	PC[27, 28], PS[67]
9-Hydroxy-12-oxo- 10-dodecenoyl		HODA-(X)	PC[27, 28], PS[67]
5-Hydroxy-8-oxo-6- octendioyl		HOdiA-(X)	PC[27, 28], PS[67]
9-Hydroxy-10- dodecenedioyl		HDdiA-(X)	PC[27, 28], PS[67]
5-Keto-8-oxo-6- octenoyl		KOOA-(X)	PC[27, 28], PS[67]

General Phospholipid Structure			
***sn*-2 Moiety**	**Structure (Y)**	**Abbreviation (C16:0 in *sn*-1)**	**Associated Head Group (X)**
9-Keto-12-oxo-10- dodecenyl		KODA-(X)	PC[27, 28], PS[67]
5-Keto-6-octendioyl		KOdiA-(X)	PC[27, 28], PS[67]
9-Keto-10- dodecenedioyl		KDdiA-(X)	PC[27, 28], PS[67]
Ester Hydrolysis			
Hydroxy		Lyso-(X)	PC[126], PE[167], PS[127]