Chapter 12
The Role of Phospholipid Oxidation Products in Inflammatory and Autoimmune Diseases

Evidence from Animal Models and in Humans

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Abstract Since the discovery of oxidized phospholipids (OxPL) and their implication as modulators of inflammation in cardiovascular disease, roles for these lipid oxidation products have been suggested in many other disease settings. Lipid oxidation products accumulate in inflamed and oxidatively damaged tissue, where they are derived from oxidative modification of lipoproteins, but also from membranes of cells undergoing apoptosis. Thus, increased oxidative stress as well as decreased clearance of apoptotic cells has been implied to contribute to accumulation of OxPL in chronically inflamed tissues.

A central role for OxPL in disease states associated with dyslipedemia, including atherosclerosis, diabetes and its complications, metabolic syndrome, and renal insufficiency, as well as general prothrombotic states, has been proposed. In addition, in organs which are constantly exposed to oxidative stress, including lung, skin, and eyes, increased levels of OxPL are suggested to contribute to inflammatory conditions. Moreover, accumulation of OxPL causes general immunmodulation and may lead to autoimmune diseases. Evidence is accumulating that OxPL play a role in lupus erythematosus, antiphospholipid syndrome, and rheumatoid arthritis. Last but not least, a role for OxPL in neurological disorders including multiple sclerosis (MS), Alzheimer's and Parkinson's disease has been suggested.

This chapter will summarize recent findings obtained in animal models and from studies in humans that indicate that formation of OxPL represents a general mechanism that may play a major role in chronic inflammatory and autoimmune diseases.

Keywords Oxidized phospholipids · chronic inflammation · autoimmune disease

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Abbreviations AMD: age-related macular degeneration; apoB: apolipoprotein B; β2/GPI: beta-2-glycoprotein; BMP: bone-morphogenic protein; DC: dendritic cell; EC: endothelial cell; HDL: high-density lipoprotein; IL: interleukin; KLF: kruppel-like factor; LDL: low-density lipoprotein; Lp-PLA_2: lipoprotein-associated phospholipase A_2; NADPH-oxidase: nicotinamide adenine dinucleotide phosphate-oxidase; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; PAPC: 1-palmitoyl-2-arachidonoyl-*sn*-3-glycero-phosphorylcholine; POVPC: 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphorylcholine; PGPC: 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine; OxPAPC: oxidized PAPC, OxPL: oxidized phospholipids; PAF: platelet-activating factor; PAF-AH: PAF-acetylhydrolase; PON: paraoxonase; PTH: parathyroid hormone; SLE: systemic lupus erythematosus; TNF: tumor necrosis factor; TLR: toll-like receptor; UV: ultraviolet.

12.1 Introduction

12.1.1 Formation of OxPL in Tissues Exposed to Oxidative Stress

Non-enzymatic oxidative modification of phospholipids in chronically inflamed tissue is mediated by free radicals which are produced by enzymes including NADPH oxidase and myeloperoxidase (Zhang et al. 2002). While ozone may play an additional role in lipid oxidation in the lung, singlet oxygen produced by ultraviolet (UV) light is the mechanism particularly relevant for skin and eye pathologies.

Polyunsaturated fatty acids and especially arachidonic acid are highly susceptible to lipid peroxidation, which leads to the generation of lipid hydroperoxides, which then 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 (Esterbauer et al. 1987). Considerable progress has been made in recent years in dissecting the molecular structures of OxPL, which consequently allowed for the experimental use of defined compounds rather than complex lipoproteins and lipid mixtures.

Oxidation of phospholipids, such as 1-palmitoyl-2-arachidonoyl-*sn*-3-glycero-phosphorylcholine (PAPC), yields a series of oxidation products (OxPAPC) some of which have been structurally identified and shown to accumulate in atherosclerotic lesions (Watson et al. 1997). Examples for structures derived from oxidation of PAPC include 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3--phosphorylcholine (POVPC) and 1-palmitoyl-2-glutaroyl-*sn*-glycero-3--phosphorylcholine (PGPC) (Watson et al. 1997), as well as a series of CD36 binding motifs, which contain an oxidatively truncated *sn*-2 acyl group with a terminal gamma- hydroxy(or oxo)-α,β-unsaturated carbonyl (Podrez et al. 2002a). The molecular properties of identified OxPL are described in detail in a recent review (Fruhwirth et al. 2007).

The exact mechanisms how OxPL induce an inflammatory response remain a matter of speculation. Since OxPL are structurally similar to some bacterial components, it is possible that these modified lipids are recognized by cells as so called "danger signals" and consequently induce an initially protective immune response involving inflammatory reactions. Oxidized moieties of phospholipids are exposed on membranes (Greenberg et al. 2008), and thereby can be recognized by "pattern recognition receptors" including scavenger receptors such as CD36 (Hazen and Chisolm 2002). Due to their structural similarity to platelet activating factor (PAF), fragmented alkyl-OxPL ("PAF-like lipids") serve as specific agonists for the PAF receptor (PAF-R). It was shown that PAF-like lipids derived from oxidized LDL act on cells that express the PAF-R at low concentrations (Marathe et al. 1999, 2002; Smiley et al. 1991; Zimmerman et al. 2002).

Initially, the formation of lipid oxidation products has been considered solely detrimental, since it was demonstrated that these toxic products propagate inflammation and tissue damage. Only recently it became evident that certain OxPL also exert antiiflammatory, tissue protective effects (Bochkov 2007). This is the case when cells and tissues respond towards these oxidatively modified stress signals with upregulation of protective genes. In addition, OxPL derived from arachidonic acid-containing PL potently inhibit effects of lipopolysaccharide (LPS) (Bochkov et al. 2002a). Therefore, development of pharmacological agents based on structures of OxPL has been implied, and various treatment scenarios such as in lung disease and in sepsis have been suggested.

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. Among those diseases are cardiovascular disease, diabetes and its complications including eye and kidney diseases, but also general immune modulation that could play an important role in sepsis and autoimmune diseases. Furthermore, evidence is accumulating that OxPL may play a role in neurological disorders including Alzheimer's and Parkinson's disease (Fig. 12.1).

12.1.2 Catabolism of OxPL

OxPL have to be promptly removed *in vivo* in order to diminish adverse biological effects that may arise through their uncontrolled accumulation. One of the candidate enzymes responsible for the hydrolysis of OxPL is PAF-acetylhydrolase (PAF-AH), also termed lipoprotein associated phospholipase A_2 (Lp-PLA2) (Dada et al. 2002; Zalewski et al. 2006; Khuseyinova et al. 2005). PAF-AHs were originally identified as enzymes that hydrolyze the acetyl group at the *sn*-2 position of PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine). Oxidation of the *sn*-2 acyl group of a phospholipid results in aldehydic or

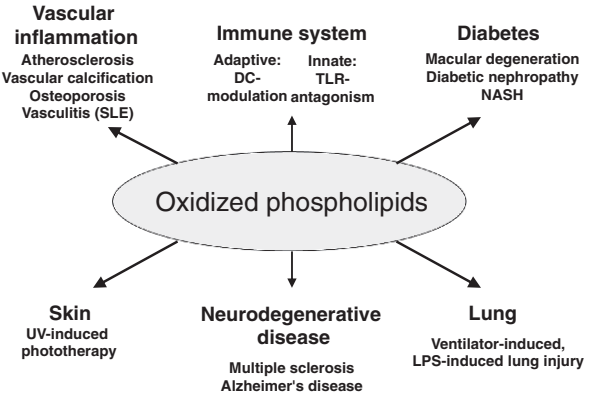

Fig. 12.1 Oxidized phospholipids play a role in pathologies that have been associated with oxidative stress. DC, dendritic cell; LPS, lipopolysaccharide; NASH, nonalcoholic steatohepatitis; SLE, systemic lupus erythematosus; TLR, toll-like receptor; UV, ultraviolet

carboxylic groups (as in POVPC or PGPC, respectively), formation of which increases the substrate affinity for plasma PAF-AH. Moreover, oxidatively modified, non-fragmented phospholipids such as esterified F2-isoprostanes and phospholipid hydroperoxides are good substrates for PAF-AH. Consequently, PAF-AH catabolizes PAF as well as OxPL in oxidized LDL (OxLDL). It is assumed that hydrolysis OxPL by PAF-AH limits the activity of these proatherogenic lipids. Indeed, overexpression of PAF-AH in cells or tissues was shown to suppress oxidative stress-induced cell death. Adenovirus-mediated overexpression of PAF-AH in rabbits resulted in reduced foam cell formation (Turunen et al. 2004). Moreover, recombinant plasma PAF-AH was effective in treating acute pancreatitis, asthma and anaphylactic shock in animal models (Arai et al. 2002). Many recent studies have correlated PAF-AH (Lp-PLA2) activity with severity of vascular disease and atherosclerosis (Tsimikas et al. 2007). The role of PAF-AH in atherogenesis is not quite clear yet; the degradation of OxPL may be protective, however, accumulation of the enzymatic products lyso-PC and fatty acids may contribute to disease progression.

Paraoxonase (PON) is an enzyme associated with HDL and was shown to degrade OxPL (Mackness et al. 1998b, 2006b; Mackness and Durrington 1995b; Marathe et al. 2003). Beneficial effects of degradation of OxPL by PON are demonstrated by studies where overexpression of PON protected against atherosclerosis, while PON deficiency led to increased lesion formation (Ng et al. 2005; Shih et al. 1998, 2000; Tward et al. 2002; Watson et al. 1995). A recent study shows that PON overexpression inhibits intimal hyperplasia (Miyoshi et al. 2007).

Gene polymorphisms in both PAF-AH and PON have been correlated with the incidence of atherosclerosis in humans (Agachan et al. 2004; Ahmed et al.

2001; Bilge et al. 2007; Cao et al. 1998; Dessi et al. 1999; Fortunato et al. 2003; Arai et al. 2002; Kakafika et al. 2003; Yamada et al. 2000).

12.2 OxPL in Vascular Disease

12.2.1 Atherosclerosis

Atherosclerosis is characterized by chronic inflammation of large arteries with clinical consequences including myocardial infarction and stroke. Although the knowledge about the mechanisms underlying atherosclerosis and its complications has dramatically increased, the question about the initiating factors of atherogenesis remains unsolved.

There is extensive evidence that accumulation and subsequent oxidative modification of LDL particles in the subendothelial space play a key role in development and progression of atherosclerosis (Lusis 2000; Berliner et al. 1995; Leitinger 2005). Phospholipid oxidation products are found at high concentrations within fatty streak lesions of cholesterol fed rabbits, mice, and in human atherosclerotic lesions (Watson et al. 1997; Berliner et al. 2001; Subbanagounder et al. 2000; Subbanagounder et al. 2000; Huber et al. 2002). Antibodies against OxPL are present in the serum of apoE-deficient mice and the presence of antibodies against OxPL in patients with atherosclerosis, diabetes, hypertension and other chronic inflammatory diseases further underlines the importance and potential functional relevance of these molecules (Binder et al. 2005).

OxPL are thought to play an essential role in various settings and stages of atherosclerotic lesion initiation and progression. OxPL were shown to activate endothelial cells to specifically bind monocytes, an initiating step in the development of atherosclerotic lesions (Huber et al. 2006; Berliner et al. 1990; Leitinger et al. 1999). Moreover, OxPL may compromise the barrier properties of the vascular endothelium, which may facilitate transmigration of LDL and inflammatory cells into the subendothelial layers. In this context, it was demonstrated that OxPAPC alters the expression, phosphorylation, and localization of tight junction proteins, such as occludin (DeMaio et al. 2006). On the other hand, endothelial barrier function was shown to be enhanced by certain OxPL via activation of Cdc42 and Rac (Birukov et al. 2004). Recently, it was shown that OxPL potently affect connexin expression and function in endothelial (EC) and smooth muscle cells (SMC) of the vascular wall (Isakson et al. 2006) which may have significant effects on the progression of atherosclerotic lesions (Chadjichristos and Kwak 2007; Kwak et al. 2003).

OxPL also potently activate vascular SMC and macrophages, resulting in changes of gene expression profiles and phenotypes of the respective cell type. Several papers have reported that OxPL, including POVPC, stimulated increased cell division and possibly differentiation of SMC (Heery et al. 1995;

Chatterjee et al. 2004), while others showed activation of apoptotic signaling pathways (Loidl et al. 2003). Recently, it was shown that POVPC caused phenotypic switching in SMC by suppressing the expression of multiple KLF4-dependent differentiation markers and induction of proinflammatory gene expression (Pidkovka et al. 2007).

Finally, OxPL are implicated to control end stage disease consequences including plaque rupture. There is evidence that OxPAPC activates alkaline phosphatase in calcifying vascular cells, suggesting a role for OxPL in vascular calcification (Parhami et al. 1997). We have recently shown that OxPL may increase the propensity of atherosclerotic lesions towards rupture by upregulation of matrix metalloproteinases and by inducing angiogenesis (Bochkov et al. 2006), both of which may contribute to destabilization of advanced plaques.

Further evidence for a role of OxPL in atherogenesis *in vivo* comes from studies showing that knocking out or inhibiting putative receptors for OxPL (including the PAF receptor, CD36, and toll-like receptors (TLRs) 2 and 4) leads to a decrease in experimental atherogenesis (Berliner and Watson 2005; Subbanagounder et al. 1999; Febbraio et al. 2000; Mullick et al. 2005; Tobias and Curtiss 2005). Moreover, when OxPAPC was directly applied to murine carotids *in vivo* using a pluronic gel, a pattern of inflammatory genes was upregulated, similar to that seen in experimental atherosclerosis (Furnkranz et al. 2005). Taken together, these studies clearly demonstrate that OxPL contribute to atherogenesis through their effects on vascular wall cells influencing several functions that are important for the initiation, progression and end stage events including plaque rupture.

12.2.2 Plasma Levels of OxPL as Cardiovascular Risk Markers

There is extensive evidence that changes in plasma OxPL/apoB ratios, measured using the murine monoclonal antibody E06 (Tsimikas 2006b; Tsimikas and Witztum 2001) may reflect the extent of atherosclerotic disease burden (Tsimikas et al. 2005, 2006). It was shown that OxPL/apoB levels are increased in patients with coronary, carotid or femoral artery disease, acute coronary syndromes and after percutaneous coronary intervention (Tsimikas et al. 2006).

Moreover, *Tsimikas et al.* examined whether plasma OxPL/apoB levels may indicate the vessel wall content of OxPL during atherosclerosis progression or regression. They used cynomolgus monkeys and New Zealand White rabbits and OxPL content was measured in plasma and immunohistochemically in aortic plaques at baseline, after a high-fat/high-cholesterol diet and after reversion to normal chow. Immunostaining revealed that during atherosclerosis progression OxPL co-localized with apoB-100, whereas during regression OxPL virtually disappeared. These data suggest that changes in plasma levels

of OxPL/apoB ratios reflect changes in OxPL content in atherosclerotic plaques during dietary-induced atherosclerosis progression and regression (Tsimikas et al. 2007).

In humans, OxPL were shown to circulate on apoB-containing lipoproteins, primarily on Lp(a) (Tsimikas et al. 2005, 2006a). Circulating levels of OxPL were strongly associated with angiographically documented coronary artery disease, and data from these studies suggest that the atherogenicity of Lp(a) may be mediated in part by associated OxPL (Tsimikas et al. 2005). Data obtained from the Bruneck study demonstrate that the OxPL/apoB levels predict 10 year CVD event rates independently of traditional risk factors such as hsCRP (Kiechl et al. 2007). Increasing Lp-PLA2 activity was shown to further amplify the risk of CVD mediated by oxPL/apoB (Tsimikas et al. 2006). Thus, OxPL/apoB and Lp-PLA2 levels can be used to predict symptomatic cardiovascular disease and even new cardiovascular events.

12.2.3 OxPL as Link Between Atherosclerosis and SLE

Associations of several autoimmune diseases with atherosclerosis have been observed and a role for LDL oxidation especially in systemic lupus erythematosus (SLE) has been suggested (Frostegard et al. 2005; Hayem et al. 2001; Svenungsson et al. 2001). OxLDL forms immune complexes with β2GPI, which can be detected in the plasma of patients. OxLDL/β2GPI complexes have been demonstrated in patients with syphilis, infectious endocarditis, diabetes mellitus, antiphospholipid syndrome and chronic nephritis, indicating that oxidation of LDL and the formation of complexes with β2GPI is not restricted to SLE. It is hypothesized that these autoantibodies accelerate the development of atherosclerosis in autoimmune patients.

Further evidence for OxPL as a link between atherosclerosis and lupus comes from a recently described new mouse model (Feng et al. 2007). These authors created double knockout apoE2/2Fas2/2 mice, which spontaneously develop lupus-like disease, increased atherosclerotic lesions, accompanied by decreased bone density. Interestingly, apoE2/2Fas2/2 mice had decreased serum OxPL on apoB-100-containing particles but an increase in serum IgG antibodies to OxPL. Serum IgG antibodies to OxPL correlated positively with glomerular tuft areas and aortic lesion areas (Feng et al. 2007). It was presviously shown that IgG autoantibodies to OxPL correlated with aortic lesion areas in apoE-deficient mice, and therefore were considered atherogenic, while IgM antibodies that recognize phosphorylcholine in OxPL and OxLDL seem to be protective against atherosclerosis (Binder et al. 2002). These data provide evidence that IgG autoantibodies to OxPLs and immune complexes are important contributors to atherosclerosis and autoimmune diseases including SLE and glomerulonephritis, likely triggering shared pathways that promote the pathogenesis of these diseases.

12.2.4 OxPL as Link Between Atherosclerosis and Osteoporosis

Atherosclerosis has been associated with reduced bone mineral density and fracture risk and effects of high fat diet on bone formation have been reviewed (Parhami et al. 2001). It was demonstrated that OxPAPC inhibits spontaneous osteogenic differentiation of marrow stromal cells and mineralization of calvarial preosteoblasts, suggesting that OxPL may account for the clinical link between atherosclerosis and osteoporosis (Huang et al. 2007). Moreover, OxPAPC attenuated induction of osteogenic markers alkaline phosphatase and osteocalcin, as well as expression of PTH receptor by BMP-2. It was further shown that OxPAPC affected osteogenic signaling by inhibiting PTH signaling. Since anabolic agents that promote bone formation are increasingly used as treatment for osteoporosis, these data also suggest that OxPL may interfere with anabolic therapies for osteoporosis (Huang et al. 2007).

In the apoE2/2Fas2/2 mouse it was shown that serum levels of IgG autoantibodies to OxPL, which positively correlated with aortic lesion areas and glomerular tuft areas, correlated negatively with bone density, indicating that IgG anti-OxPL may also be involved in the process of osteoporosis. Interestingly, osteoporosis has been observed more frequently in SLE patients with cardiovascular disease, which is associated with increased OxLDL and autoantibodies to OxLDL (Svenungsson et al. 2001). The precise mechanism of osteopenia in association with atherosclerosis and the potential role of OxPL and their autoantibodies in bone loss require further investigation.

12.2.5 Platelets and Thrombosis

Activation of the endothelium leads to a prothrombotic phenotype. We have shown that OxPL stimulate production of tissue factor in cultured human EC (Bochkov et al. 2002b), an effect that could be inhibited by genestein (Holzer et al. 2007). The prothrombotic phenotype in EC is further enhanced by inhibition of the anticoagulant activity of tissue factor pathway inhibitor (Hiraishi et al. 2002) and downregulation of the anticoagulant glycoprotein thrombomodulin (Ishii et al. 2003). Collectively, these findings demonstrate that OxPL promote a transition from an anticoagulant endothelium to the procoagulant state such as seen in hyperlipidemia.

Hyperlipidemia is also associated with enhanced platelet reactivity. Activated platelets adhere to the endothelium and release vasoactive mediators which induce vasoconstriction and endothelial dysfunction. While preincubation of platelets with OxPAPC did not induce platelet aggregation, it resulted in increased surface expression of CD62p and CD41. Moreover, binding of an anti-CD36 Ab was inhibited, indicating that platelet CD36 is a main receptor responsible for binding of oxidized lipoproteins (Hartwich et al. 2002). Podrez

et al. demonstrated that the prothrombotic platelet phenotype in hyperlipidemia was mediated by CD36. Genetic deletion of *Cd36* was protective against enhanced platelet reactivity, and OxPL were able to bind and activate platelets via CD36. Thus, interactions of platelet CD36 with specific endogenous OxPL play a crucial role in the well-known clinical associations between dyslipidemia, oxidant stress and a prothrombotic phenotype (Podrez et al. 2007). Direct platelet-activating activity by OxPL may also be induced by OxPL that structurally mimic PAF ("PAF-like lipids") (Marathe et al. 1999, 2002; Smiley et al. 1991; Zimmerman et al. 2002).

Furthermore, OxPL were shown to modulate the activity of the platelet prothrombinase complex, a major contributor to overall thrombin formation. Platelet-dependent thrombin generation was induced by ethanolamine phospholipids (PE) present in oxidized LDL. It was shown that oxidation products of unsaturated diacyl-PE were mainly responsible for the increased prothrombinase activity and synthetic aldehyde-PE adducts largely reproduced the stimulation of the thrombin generation (Zieseniss et al. 2001). These data suggest that oxidized PE contribute to the prothrombotic phenotype by increasing prothrombinase activity in platelets.

Recently, it was shown that protein C inhibitor (PCI) interacted with different phospholipids and their oxidized forms, thereby increasing its activity (Malleier et al. 2007). The protein C system represents a major anticoagulant pathway. PCI, a member of the serpin (serine protease inhibitor) family of protease inhibitors, was originally described as an inhibitor of the anticoagulant serine protease activated protein C. These results suggest that phospholipids are important endogenous cofactors of PCI and stimulation of PCI activity by OxPL could therefore increase the risk for thrombotic events.

12.3 Diabetes and Associated Diseases

12.3.1 Type II Diabetes

Oxidative tissue damage, as evidenced by increased production of oxidized lipids has been reported in type II diabetes (Sampson et al. 2002). Moreover, the degradation of OxPL was shown to be defective in type II diabetic patients, possibly due to decreased PON activity on the HDL from these patients. In this study the authors compared HDL from three different populations, controls, patients with type II diabetes and patients with cardiovascular disease. The data demonstrated that the ability of HDL from patients with cardiovascular disease but also from type II diabetic patients without cardiovascular disease to degrade OxPAPC *in vitro* was significantly reduced (Mastorikou et al. 2006). This was accompanied by significantly higher levels of circulating plasma oxidized LDL. The authors conclude that increased OxPL levels could

contribute to the increased susceptibility of type II diabetic patients to develop cardiovascular disease.

Furthermore, serum PON 55 and 192 polymorphsms and PON activity have been implicated to correlate with incidence of non-insulin dependent DM and also in type II diabetes complicated by retinopathy (Mackness et al. 1998a, 2005, 2006a; Mackness and Durrington 1995a; Sampson et al. 2005). Although it remains to be shown whether OxPL contribute to adipose tissue inflammation and insulin resistance, these results imply a role for OxPL in the development of complications associated with type II diabetes.

12.3.2 Eye Disease

One of the complications associated with type II diabetes is eye disease and several studies have also shown an association of macular degeneration and atherosclerosis (Mullins et al. 2000; Friedman 2000). In that context, it was suggested that PON gene polymorphisms and plasma OxLDL levels may be risk factors for age-related macular degeneration (Ikeda et al. 2001).

Oxidative modification of phospholipids has been implicated in various pathologies of the eye. Lipid oxidation was postulated as the underlying pathologic trigger in age-related macular degeneration (AMD), cataractogenesis (reviewed in (Huang et al. 2006)) and the association between lens opacification and lipid oxidation has been demonstrated. Oxidation of phospholipids in the eye may be initiated by singlet oxygen produced by UV light, which acts on lens epidermal cells and causes oxidative membrane damage. Due to the lack of cell turnover in the lens, the prolonged exposure to oxidative stress results in compositional changes greater than those reported in any other organ (Huang et al. 2006).

A study using human donor eyes showed that OxPL were present in the photoreceptors and retinal pigment epithelium of the normal human macular area, and their levels increased with age. Using the monoclonal antibody DLH3 against OxPL (Itabe et al. 1994), the authors demonstrate that eyes with AMD showed more intense immunoreactivity than age-matched normal eyes (Suzuki et al. 2007). These findings suggest that oxidative stress is involved in the pathogenesis of AMD, possibly by oxidizing phospholipids in the photoreceptors. Thus, it is conceivable that controlling oxidation of phospholipids may be a potential treatment for AMD.

Studies by Hoppe et al. show that OxLDL as well as oxidized lipid-protein complexes inhibit processing of photoreceptor outer segments and alter phagosome maturation in retinal pigment epithelium (Hoppe et al. 2001, 2004a, b; Sun et al. 2006). Clearance of shed photoreceptor outer segments by the retinal pigment epithelium, a tissue with one of the highest turnover rates in the body, is critical to the maintenance and normal function of the retina. It was shown

that OxPL may serve as endogenous ligands mediating the uptake of photoreceptor outer segments via the scavenger receptor CD36. OxPL, that contained a CD36 recognition motif, were formed *in vivo* in the retinas of dark-adapted rats following physiological light exposure. These studies suggest that intense light exposure initiates oxidative modification of PL in photoreceptor outer segments, necessary for CD36-mediated phagocytosis under oxidant stress conditions (Sun et al. 2006).

CD36 was also shown to be essential for inhibiting angiogenesis when activated by thrombospondin-1. Consequently, CD36 and thrombospondin-1 were shown to be involved in mediating antiangiogenic signals in ischemic proliferative retinopathy (Mwaikambo et al. 2006). These authors report that expression of CD36 in macrophages and microvascular endothelial cells after corneal injury suppresses corneal angiogenesis. They further show that POVPC inhibited neovessel formation, which could be blocked by an antibody recognizing CD36. POVPC also diminished VEGF-A expression and induced regression of newly grown vessels. These data indicate that certain OxPL species may negatively affect corneal neovascularisation.

12.3.3 Kidney

Diabetic nephropathy consequently results in chronic renal insufficiency and need for dialysis. End-stage renal failure is accompanied by increased oxidative stress and patients are at increased risk to develop cardiovascular disease. Oxidatively modified LDL was shown to be localized in kidneys (Exner et al. 1996) and a role for OxPL in kidney disease was suggested.

Evidence for a role of OxPL in kidney disease comes from studies in mouse models where infusion of an apoE-derived peptide (D-4F) lowered the presence of glomerular and tubulo-interstitial OxPL resulting in reduced renal inflammation (Buga et al. 2008). Evidence that OxPL could be involved in kidney disease in humans comes from a recent study demonstrating that OxPL serve as biomarkers in patients with end-stage renal failure (Bossola et al. 2007). When the effect of hemodyalysis on OxPL levels was analysed, the results demonstrated a significant reduction in OxPL/apoB following dialysis, despite the prooxidant effects of the procedure.

IgG antibodies to OxPL may also contribute to renal complications, especially in glomerulonephritis, where anti-dsDNA antibodies were shown to play a role. Moreover, autoantibodies to other oxidation-specific epitopes, including malondialdehyde, have been suggested to participate in a model of human membrane nephropathy (Dominguez et al. 2000). In the apoE2/2Fas2/2 mouse, increased IgG deposition in glomeruli with increased IgG anti-POVPC and anti-PGPC suggested that IgG anti-OxPL might act similarly to IgG anti-dsDNA to induce nephritis (Feng et al. 2007).

12.3.4 Liver

Nonalcoholic fatty liver disease (NAFLD) is a growing hepatological problem in Western countries. NAFLD may be limited to the fatty liver alone, or it may progress to nonalcoholic steatohepatitis (NASH) (Alisi and Nobili 2007). Although the etiology of NASH is unknown, it is frequently associated with obesity, type II diabetes mellitus, and hyperlipidemia. The pathogenesis of NASH is not yet fully understood, but it is widely accepted that insulin resistance is a culprit leading to hepatic lipid accumulation followed by oxidative stress, inflammation and finally necrosis. These modifications enhance lipid peroxidation, hepatocyte injury resulting in inflammation and fibrosis, and may evolve into cirrhosis and hepatocellular carcinoma.

OxPL have been found in fatty liver, and their levels were increased especially in NAFLD (Ikura et al. 2006), implicating a role for OxPL in disease progression. Evidence for OxPL as important contributors to liver injury comes from a study demonstrating that PAF-AH (II)-deficient mice were no longer protected against oxidative stress-induced hepatic injury (Kono et al. 2008). Cells derived from PAF-AH (II)-deficient mice were more sensitive to oxidative stress than those derived from wild-type mice and treatment of PAF-AH (II)-deficient mice with carbon tetrachloride demonstrated a delay in hepatic injury recovery, which correlated with increased production of OxPL. These results indicated that PAF-AH (II) metabolizes OxPL thereby protecting liver tissue from oxidative stress-induced injury.

NAFLD has become one of the critical chronic liver diseases worldwide. There is a great urgency to clarify the pathogenesis of NAFLD/NASH to establish reasonable treatment strategies (Alisi and Nobili 2007). Further understanding of the role of OxPL in NAFLD/NASH may lead to development of such strategies.

12.4 Role of OxPL in Immune Response

12.4.1 Innate Immune System

In chronically inflamed tissue, the local immune response is determined by environmental factors, such as accumulating lipid oxidation products. Phospholipid oxidation products resemble danger signals, which accumulate under conditions of increased oxidative stress and cell death, and as such were shown to act as endogenous regulators of the innate immune response (Binder et al. 2002, 2003; Bochkov et al. 2002a). Upon oxidative modification, phospholipids are recognized by certain pattern recognition receptors (PRRs), indicating the formation of structural motifs, which are sensed by the innate immune system as danger signals or “altered-self”. PRRs that have been implicated in the

recognition of OxPL include scavenger receptors such as CD36, TLRs, CD14, LPS-binding protein and C-reactive protein (Miller et al. 2003a; Walton et al. 2003b; Chang et al. 2002; Hazen and Chisolm 2002; Podrez et al. 2002b; Boullier et al. 2005; Miller et al. 2003b). The fact that the oxidation process renders phospholipids "visible" to the innate immune system bears important implications for the pathogenesis of both chronic inflammatory and autoimmune diseases (Kronke and Leitinger 2006).

Further evidence for recognition of endogenous phospholipid oxidation products by PRRs comes from our studies demonstrating that OxPAPC competes with LPS for the binding to PRRs such as LPS-binding protein and CD14. In this case oxidized phospholipids act as endogenous "LPS receptor-antagonists" and block LPS-induced signaling events including the activation of the pro-inflammatory transcription factor NFκB (Bochkov et al. 2002a). This protective role of phospholipid oxidation products during an acute LPS-induced inflammation may represent an important feedback mechanism whereby accumulating oxidized phospholipids limit further tissue-damage by blocking the innate immune response.

Based on findings that OxPL inhibit LPS-induced inflammation, possible therapeutic effects of OxPAPC in rodents with acute necrotizing pancreatitis were investigated. In this study, treatment with OxPAPC decreased the severity of experimental pancreatitis in mice and rats and the protective effect of OxPAPC was mediated, at least in part, through blocking the LPS signaling pathway (Li, Wang, and Wu 2007).

12.4.2 Endotoxemia and Sepsis

We and others have shown previously that OxPL effectively inhibit the activation of TLR4 (Bochkov et al. 2002a; Walton et al. 2003a; Eligini et al. 2002), the mechanism of which may involve inhibition of interaction of LPS with LBP, CD14. The biological significance of this finding is underlined by studies demonstrating that the exogenous administration of OxPAPC could prevent mortality of mice exposed to high doses of LPS (Bochkov et al. 2002a). Thus, the formation of OxPL seems to inhibit LPS-induced inflammation *in vivo* and may be protective in settings of endotoxic shock.

Based on these findings, Knapp et al. investigated the effects of OxPL during *E. coli*-induced abdominal sepsis *in vivo*. In contrast to the protective effects observed in endotoxemia, administration of OxPAPC rendered mice highly susceptible to *E. coli* peritonitis, as indicated by an accelerated mortality and enhanced bacterial outgrowth and dissemination. The mechanism by which OxPAPC impaired the immune response was by diminishing phagocytosing capacity of neutrophils and macrophages. These data suggest that OxPL arising as a byproduct of the respiratory burst during acute inflammation may contribute to mortality during Gram-negative sepsis *via* impairment of the

phagocytic properties of professional phagocytes involved in innate immunity (Knapp et al. 2007).

Although OxPL generated at sites of inflammation might be able to prevent overwhelming inflammation in settings of sterile inflammation, impairment of the innate immune response to bacterial infections by OxPL is detrimental for the outcome of host defense reactions.

12.4.3 Adaptive Immune System

12.4.3.1 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 OxLDL are present in the plasma of humans and animals and their titers have been shown to correlate with atherosclerosis progression (Horkko et al. 2000; Tsimikas et al. 2001; Cyrus et al. 2001), as well as in several autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis (Amara et al. 1995; Cvetkovic et al. 2002; Hayem et al. 2001; Wu and Rao 1999; Tsimikas et al. 2005). 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 (Palinski et al. 1996; Horkko et al. 1999; Chang et al. 1999).

12.4.3.2 OxPL as Regulators of Dendritic Cell (DC) Maturation

Recent *in vitro* and *in vivo* evidence highlights the impact OxPL exert on the maturation process of DC, where these molecules mediate both stimulatory and inhibitory effects, thereby influencing decisive steps of the adaptive immune response. Studies performed by our lab have shown that phospholipid oxidation products such as OxPAPC inhibit basic steps of the classical DC maturation process. For instance, OxPAPC blocks LPS-induced expression of costimulatory molecules including CD40 and CD83 and inhibits secretion of pro-inflammatory cytokines such as IL-12 and TNF. In parallel with the inhibitory influence on DC maturation, OxPAPC treatment of DCs dampened DC-induced T-cell proliferation as well interferon-γ secretion by T-cells (Bluml et al. 2005). This reduction in interferon-γ levels in T-cells, together with the inhibition of IL-12 expression in DCs, indicates a specific block of the Th1-orientated immune response by OxPAPC.

Important evidence for a role of OxPL as modulators of DC function *in vivo* comes from studies performed in ApoE-deficient mice, which have elevated levels of OxLDL and suffer from severe dyslipidemia and accelerated

atherosclerosis. ApoE-deficient mice show a severely compromised immune response, resulting in a depressed delayed-type hypersensitivity reaction (Laskowitz et al. 2000; Ludewig et al. 2001). It was shown that these mice exhibit an altered DC function, indicated by impaired migratory capabilities of skin DCs. Elevated levels of PAF and PAF-like OxPL caused these immunological alterations in ApoE-deficient mice. Consequently, the treatment with recombinant PAF-AH restored normal immunological parameters in these mice (Angeli et al. 2004).

Using ApoE-deficient mice on a high-fat/cholesterol diet, Shamshiev et al. showed that dyslipidemia inhibited TLR-induced production of proinflammatory cytokines, including IL-12, IL-6, and TNF, as well as up-regulation of costimulatory molecules by CD8α(–) DCs, but not by CD8α(+) DCs, *in vivo*. Decreased DC activation profoundly influenced Th cell responses, leading to impaired Th1 and enhanced Th2 responses. As a consequence of this immune modulation, host resistance to Leishmania major was compromised (Shamshiev et al. 2007).

These results show that a dyslipidemic microenvironment can directly interfere with DC responses to pathogens and skew the development of T cell-mediated immunity. It will be important to specify the molecular structures within these phospholipid oxidation products that are responsible for their effects on DC function. This will eventually allow designing low molecular substances, which mimic their immunomodulatory effects.

12.5 Lung

OxPL are constantly formed and accumulate in murine lung tissue (Nakamura et al. 1998) and in the lung circulation as a result of increased oxidative stress that accompanies pathological conditions such as acute lung injury, lung inflammation, acute respiratory distress syndrome (ARDS), ventilator-induced lung injury (VILI), systemic inflammatory response syndrome (SIRS) and sepsis. Under these conditions, lung vascular barrier function is largely compromised.

Interestingly, studies by Birukov et al. and other reports strongly suggest barrier-protective effects of certain OxPL on human pulmonary endothelium (Birukova et al. 2007). It was shown that OxPL produce a sustained increase in transendothelial electrical resistance of human pulmonary EC and restore barrier disruption induced by edemagenic agonist thrombin *in vitro* (Birukov et al. 2004). Subsequently it has been shown that intravenously injected OxPAPC, but not unoxidized PAPC, protects rats from lung inflammation and injury induced by intratracheal application of LPS (Nonas et al. 2006). Measurements of endothelial transmonolayer electrical resistance and immunofluorescent analysis of monolayer integrity demonstrated that OxPAPC markedly attenuated LPS-induced tissue inflammation, barrier disruption,

and cytokine production (Nonas et al. 2006). These studies demonstrate protective effects of OxPL on LPS-induced lung dysfunction *in vivo* and *in vitro*. Evidence for protective effects of OxPL in the lung being independent of their LPS-antagonizing properties comes from a recent study that demonstrates that OxPL are also protective in ventilator-induced lung injury (Nonas et al. 2008). Thus, OxPL, which are constantly present in the lung (Uhlson et al. 2002), have a potential to preserve function of lung during life-threatening systemic inflammation as well as in settings of non-infectious lung injury.

12.6 Skin

Long wave ultraviolet (UV) irradiation causes oxidizing stress to the skin that provokes synthesis of antioxidant stress response genes. UVA1 (340–400 nm) is frequently used in clinical UV therapy for inflammatory skin diseases such as psoriasis (Legat et al. 2004a, b), morphea (Gruss et al. 2001), atopic dermatitis, scleroderma and graft versus host disease (Mang and Krutmann 2005). Especially cells in the dermis respond to UVA1 irradiation by inducing synthesis of protective stress response genes that have anti-inflammatory effects. The generation of singlet oxygen by UVA1 (340–390 nm) leads to subsequent oxidation of intracellular membrane lipids. Strong evidence suggests that oxidation of membrane lipids by UVA1 is involved in response gene induction (Baier et al. 2007; Basu-Modak and Tyrrell 1993; Basu-Modak et al. 1996). UVA irradiation also perturbs the activity of the immune system (Furio et al. 2005). However, underlying mechanisms and structures of responsible lipid oxidation products are poorly understood.

Lipid mediators that are formed upon UV irradiation are classically regarded as inducers and propagators of chronic inflammatory reactions, but recently their role is being redefined since data have accumulated that demonstrate their potential to induce anti-inflammatory genes, to prevent expression of pro-inflammatory genes, and to interfere with the function of professional antigen presenting cells (Nonas et al. 2006; Gruber et al. 2007; Bochkov and Leitinger 2003). Phospholipid mediators that arise upon UV irradiation are fragmented PAF-like lipids, that can elicit immuno-suppressive effects in the skin (Walterscheid et al. 2002), as well as long chain acyl-OxPL, that were shown to induce HO-1 expression (Kronke et al. 2003), which, besides being a major antioxidant response gene, itself has immunomodulatory effects (Allanson and Reeve 2004). Investigating the formation and biological properties of OxPL in skin cells after UVA-1 irradiation, we found that the common membrane phospholipid PAPC is oxidized by UVA1 *in vitro* and in living dermal fibroblasts, the major UVA1 responsive dermal cell type. We have shown that UVA1-OxPL regulate synthesis of stress response enzymes in cultured skin cells and in cells of the immune system (Gruber et al. 2007; Ishikawa et al. 1997).

These results show that certain OxPL species may mediate anti-inflammatory and immunomodulatory effects in the skin, particularly after UV irradiation.

12.7 Neurological Disorders

It has been long known that oxidative modification of membrane lipids accompanies neurological tissue damage, which may ultimately lead to multiple sclerosis (MS), Parkinson's and Alzheimer's disease. The formation of phospholipid hydroperoxides was demonstrated in rat brain synaptosomes, a process that could be inhibited by alpha-tocopherol (Shi et al. 1999). Moreover, oxidative modification in membrane phospholipids of the central nervous system was detected after chemotherapy (Miketova et al. 2005). Recently, the same family of oxidized choline glycerophospholipids that previously has been identified in atheroma to serve as endogenous ligands for the scavenger receptor CD36, has been found in the brain. A subset of these OxPL possessing *sn*-2 esterified fatty acyl hydroxyalkenal groups, can undergo intramolecular cyclization and dehydration to form a terminal furyl moiety (oxPC-furan). Generation of oxPC-furans was demonstrated in brain tissues following cerebral ischemia (Gao et al. 2006).

OxPL, including the aldehyde-containing POVPC, were detected in the brain of MS patients. MS is an inflammatory neurodegenerative autoimmune disease, which involves formation of plaques of demyelination in the brain, eventually resulting in axonal degeneration. In brains from MS patients, E06-positive areas were present in MS plaques, which also showed evidence of OxPL-modified proteins, while E06 reactivity was largely absent from control tissue. Moreover, spinal cords from mice trated to develop experimental allergic encephalomyelitis also showed strong immunoreactivity for OxPL (Qin et al. 2007). These authors conclude that the formation of OxPL could play a role in the progression of MS.

Investigation of phospholipid catabolic and anabolic enzymes revealed lower activity of PLA_2 and phosphoethanolamine- and phosphocholine-cytidylyltransferases in autopsied substantia nigra of patients with idiopathic Parkinson's disease. The decreased rate of phospholipid turnover in the pigmented neurons of the substantia nigra might result in reduced ability to repair oxidative membrane damage and thus accumulation of OxPL in Parkinson's disease (Ross et al. 2001).

Lesions of Alzheimer's disease, evident as dense plaques composed of fibrillar amyloid beta-proteins, likely develop when these proteins are first induced to form beta-sheet secondary structures. It was demonstrated that membranes containing oxidatively damaged phospholipids accumulated amyloid beta-protein significantly faster than membranes containing unoxidized phospholipids. The protein on oxidized membranes more readily changed conformation to a beta-sheet, indicating that oxidatively damaged phospholipid membranes

promote beta-sheet formation by amyloid beta-proteins. These data suggest a possible role for lipid peroxidation in the pathogenesis of Alzheimer's Disease (Koppaka et al. 2003; Koppaka and Axelsen 2000).

12.8 Concluding Remarks

The formation of biologically active phospholipid oxidation products represents a general concept that occurs in all inflamed and stressed tissues. The relative abundance of individual structures, as well as presence of recognition systems for these "danger signals" determines cell and tissue response, which span from inflammation-propagating responses to anti-inflammatory, tissue-protective effects. Anti-inflammatory, tissue protective effects of certain structures derived from oxidative modification of phospholipids could potentially be used to develop pharmacological agents with immune-modulatory activity. Treatment strategies based on structures derived from OxPL may evolve from studies showing beneficial effects of OxPL administration in several organs and tissues, including lung, pancreas, skin but also settings of systemic inflammation including endotoxemia and sepsis. On the other hand, it has been suggested that for some drugs, the mechanism of action and possible side effects are closely linked to oxidative damage of cell membranes. A recent study shows that several antipsychotic and antineoplastic agents interact with OxPL, which may lead to modulation of their function (Mattila et al. 2007). Definitely more knowledge about pharmacodynamic properties, receptors and signaling pathways that are induced by OxPL needs to be gained in order to devise such strategies.

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