Mol. Nutr. Food Res. 2005, *49*, 1063 – 1071 DOI 10.1002/mnfr.200500086

Review

Oxidized phospholipids as triggers of inflammation in atherosclerosis

Norbert Leitinger

Department of Pharmacology and Cardiovascular Research Center, University of Virginia, Charlottesville, VA, USA

Chronic inflammatory diseases including atherosclerosis are major causes of morbidity and mortality worldwide. However, the factors, which trigger processes that determine the outcome of an inflammatory response, are still poorly understood. Accumulating evidence suggests that certain lipid oxidation products, such as oxidized phospholipids (OxPL), may represent endogenously formed factors that are capable of triggering vascular inflammation. This review will address important questions regarding mechanisms involved in acute and chronic inflammation, and discuss the role of OxPL as key players in triggering the inflammatory response in atherosclerosis. Better understanding of how OxPL contribute to vascular inflammation should lead to new strategies in the treatment of chronic inflammatory disorders.

Keywords: Atherosclerosis / Chronic inflammation / Monocyte adhesion / Oxidized phospholipids / Receptors / Signaling

Received: June 6, 2005; revised: July 28, 2005; accepted: July 29, 2005

1 Introduction

Atherosclerosis is a chronic inflammatory disease involving accumulation of lipids, lipoproteins, and mononuclear cells (monocytes and T-cells) in the subendothelial space of blood vessels [1]. Although our knowledge about the mechanisms underlying atherosclerosis and its complications has dramatically increased, the question about the initiating factors of atherogenesis remains unsolved. Accumulating evidence suggests retention of low-density lipoprotein (LDL) particles in the subendothelial space [2] with subsequent oxidative modification as key steps in atherogenesis. Recently, considerable advances have been made in dissecting the molecular components of oxidized LDL responsible for its proatherogenic effect, allowing for the experimental use of defined compounds rather than complex lipoproteins.

Oxidative modification initially gives rise to minimally oxidized LDL (MM-LDL) [3], the biological activity of which primarily results from oxidation of phospholipids such as 1-palmitoyl-2-arachidonoyl-*sn*-3-glycero-phosphorylcholine (PAPC), yielding a series of structurally defined oxidation products (OxPAPC) that accumulate in atherosclerotic lesions [4, 5]. The atherogenic potential of OxPAPC has been demonstrated in cell culture studies as shown by enhanced monocyte binding to OxPAPC-stimulated endothelial cells, concomitant with induction of MCP-1 and IL-8 [6]. However, signaling pathways involved in oxidized phospholipid (OxPL)-induced specific monocyte adhesion are not fully understood, and receptors for OxPL have not been described. Moreover, static coculture systems only incompletely model the complex cellular interactions in the vessel wall and provide no information, *e.g.*, as to whether monocyte–endothelial interactions would occur under flow. Moreover, inactivation of OxPL by protective enzymes such as paraoxonase (PON) or platelet activating factor acetyl-hydrolase (PAF-AH) may occur *in vivo*, limiting the proinflammatory potential of these lipids. Although OxPAPC has been shown to be biologically active *in vivo* when applied intravenously in mice [7, 8], the hypothesis of

Correspondence: Dr. Norbert Leitinger, Cardiovascular Research Center, University of Virginia, P.O. Box 801394, Charlottesville,VA 22908, USA
E-mail: nl2q@virginia.edu
Fax: +1-434-924-2828

Abbreviations: AA, arachidonic acid; **MM-LDL**, minimally oxidized LDL; **MPO**, myeloperoxidase; **OxPL**, oxidized phospholipids; **PAF-AH**, platelet activating factor acetyl-hydrolase, **PAPC**, 1-palmitoyl-2-arachidonoyl-*sn*-3-glycero-phosphorylcholine; **PC**, phosphatidylcholine; **PE**, phosphatidylethanolamine; **PEIPC**, epoxyisoprostane-PC; **PGPC**, 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine; **PON**, paraoxonase; **POVPC**, 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphorylcholine; **PRRs**, pattern recognition receptors; **PS**, phosphatidylserine; **TLRs**, toll-like receptors

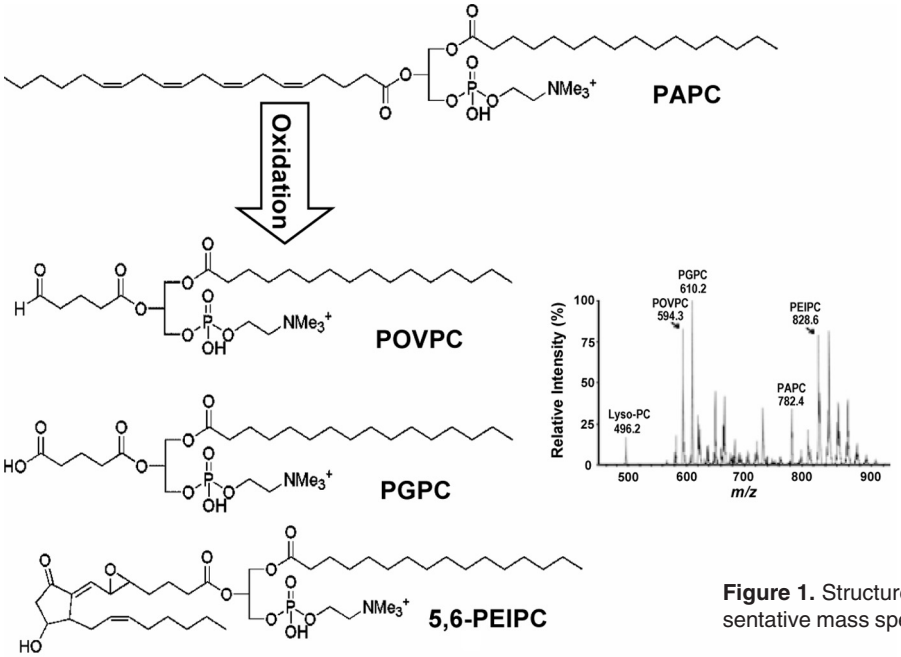

Figure 1. Structures of oxidized phospholipids and representative mass spectrogramm.

OxPL as inducers of vascular inflammation in atherosclerosis remains elusive due to the lack of data obtained in adequate animal models and it has been questioned if lipoproteins oxidized *in vitro* yield similar biological responses as lipoproteins oxidized in the arterial wall. Recently, we have shown that OxPL indeed can trigger vascular inflammation using a model where oxidized lipids are topically applied to a mouse carotid artery using Pluronic gel. This method allows for investigating the direct action of lipid oxidation products on the vascular wall mimicking accumulation of OxPL during atherosclerosis [9]. Based on the results obtained in this and previous studies, we hypothesize that specific phospholipid oxidation products trigger vascular inflammation and determine monocyte specificity characteristic of atherosclerosis and other chronic inflammatory diseases.

The purpose of this review is to discuss whether OxPL can be considered triggers of vascular inflammation, if they induce atherogenic chemokines or other inflammatory genes in the arterial wall *in vivo* and whether this would entail monocyte adhesion to the arterial endothelium.

2 Formation and structures of OxPL

Lipid oxidation is involved in the pathogenesis of various diseases including atherosclerosis, diabetes, cancer, and rheumatoid arthritis as well as in aging [10]. Oxidative modification of lipids is mediated by free radical-induced mechanisms involving enzymes such as NADPH oxidase and myeloperoxidase (MPO) [11]. Thus, at sites of inflammation, where cells generate an environment of high oxidative stress, lipid oxidation products accumulate and exert a variety of biological activities. Recent evidence suggests that biologically active OxPL accumulate at sites of inflammation, in atherogenic lipoproteins, atherosclerotic lesions, and membrane vesicles released from activated and apoptotic cells. Thus, OxPL may play an essential role in various settings of chronic and acute inflammation. Especially the involvement of OxPL in the development of atherosclerosis, which is described as a chronic inflammatory disease, is increasingly recognized.

Polyunsaturated fatty acids and especially arachidonic acid (AA) 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 [12]. Oxidation of PAPC yields oxygenated as well as fragmented products, some of which were structurally identified as 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphorylcholine (POVPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine (PGPC) [4], and epoxyisoprostane-PC (PEIPC) (Fig. 1). Meanwhile, the structures of several more biologically active lipid oxidation products have been identified [13]. Extensive oxidation of PAPC results in the formation of lysophosphatidylcholine.

3 Role of OxPL in atherogenic inflammation

3.1 OxPL induces specific monocyte adhesion

Atherosclerosis is a chronic inflammatory disease, characterized by specific infiltration of monocytes and T-cells,

while neutrophils are essentially absent in the fatty streak lesion. Previously it was demonstrated that, if stimulated with MM-LDL, endothelial cells are activated to specifically bind monocytes but not neutrophils [14]. This specificity toward mononuclear cells was subsequently observed with the activation of endothelial cells by OxPAPC [15], implying OxPL as culprits in chronic inflammation. Like MM-LDL, OxPAPC stimulated endothelial cells to specifically bind monocytes but not neutrophils. One biologically active oxidized phospholipid in OxPAPC is POVPC, which essentially mimicks the actions of MM-LDL and OxPAPC. POVPC has also been found in atherosclerotic lesions of animals and humans, and biologically active oxidized membrane vesicles [4, 16].

It has been shown that apoptosis not only leads to predominant oxidation of the membrane phosphatidylserine (PS), but also phosphatidylcholine (PC) and phosphatidylethanolamine (PE) [17]. Notably, oxidation products from PS- and PE-containing phospholipids contain homologous functional groups that result in similar biological activities. Thus, we could show that OxPAPE and OxPAPS also induced monocyte adhesion to EC [18]. The presence of oxidized PC on the surface of apoptotic cells has been demonstrated using the mAb EO6, which exclusively binds to oxidized PC [19]. It has been shown that EO6 can effectively block the uptake of apoptotic cells by macrophages. Thus, in addition to oxidized PS, the presence of oxidatively modified PC is an important signal for phagocytosis. Moreover, we showed that apoptotic blebs stimulated endothelial cells to bind monocytes but not neutrophils, whereas membrane vesicles from activated endothelial cells failed to induce monocyte binding. However, *in vitro* oxidation of these membrane vesicles from activated endothelial cells using *tert*-butyl-hydroperoxide rendered them biologically active generating POVPC. Moreover, the ability of apoptotic blebs or oxidized vesicles to induce monocyte adhesion was abolished by preincubation with the EO6 antibody recognizing oxidized PC [16]. Recently, it was shown that apoptotic blebs derived from T-cells also contain these OxPL and thus induce monocyte–endothelial interactions [20].

There are striking differences between cell activation by other proinflammatory mediators such as IL-1, TNFα, or LPS and OxPL. These mediators activate the classical NFκB pathway leading to an acute inflammatory response by elevating the expression of adhesion molecules like E-selectin, VCAM-1, or ICAM-1, resulting in adhesion of monocytes as well as neutrophils. OxPAPC, however, stimulates endothelial cells to specifically bind monocytes, but not neutrophils, a hallmark of chronic inflammation. Monocyte adhesion to endothelial cells stimulated by MM-LDL or OxPAPC is not mediated by surface expression of the adhesion molecules VCAM-1, ICAM-1, or E-selectin, but is due to deposition of CS-1-containing fibronectin on the cell surface [21]. POVPC mimicked these actions. PGPC and PEIPC, in contrast, induced endothelial surface expression of VCAM-1 and E-selectin and significantly increased both monocyte and neutrophil binding to endothelial cells, but had no effect on CS-1 expression [22]. These data provide evidence for the fact that both POVPC and PGPC are important regulators of leukocyte–endothelial cell interactions. Overriding the effects of PGPC, POVPC may play a dominant role in chronic inflammation where monocyte adhesion becomes predominant. In addition, some OxPL inhibits neutrophil binding by inhibiting E-selectin expression [18], pointing to a mechanism by which neutrophils are excluded from the fatty streak lesion [22]. The induction of monocyte binding and the inhibition of neutrophil binding involve a cAMP-coupled pathway [23]; however, the exact mechanisms of endothelial cell activation leading to specific monocyte adhesion are not known. There is evidence that LPS-induced NFκB-mediated inflammation is down-regulated by OxPAPC leading to the hypothesis that lipid oxidation products may promote the shift from an acute inflammatory response to a chronic state. However, in order to proof such a hypothesis, we need to know more about the specific inflammatory response that is elicited by oxidized lipids.

3.2 Signaling mechanisms induced by OxPL in endothelial cells

Cole *et al.* [23] showed that in HAEC, activation of αVβ1 integrins by OxPAPC was dependent on cAMP and that cAMP itself activated monocyte binding and β1 integrins *via* R-Ras activation. Activation of β1 integrins by OxPAPC was rapid and occurred within 10 min. However, maximal monocyte adhesion was observed after 4 h and required new protein synthesis. Others and we have demonstrated that activation of the MAP-kinase signaling cascade was involved in activation of endothelial cells by OxPL. Signaling mechanisms activated by OxPAPC in endothelial cells not only include elevation of cyclic AMP, rise in cytosolic Ca^{2+} levels, activation of PKA and PKC, and activation of MAP-kinase cascades, but also induction of MAP-kinase phosphatase 1 [24–26]. This results in activation of transcription mediated by Egr-1 and NFAT, CREB, PPARα, and PPARγ. Recently, we [27] have shown that OxPAPC induces the expression of HO-1 in HUVEC, involving phosphorylation of CREB, which also was dependent on MAP-kinase pathways. Others have shown that OxPL-induced endothelial activation not only involves the c-src pathway [28] and sterol-regulatory element binding protein (SREBP) [29], but also induction of COX-2 expression *via* mechanisms involving PPARγ [30].

3.3 OxPL does not activate the NFκB pathway

Inflammatory cytokines such as TNF-α or IL-1 induce expression of inflammatory genes through activation of NFκB-mediated transcription [31]. Important steps in this process are phosphorylation and degradation of the inhibitory IκBα subunit allowing translocation of active NFκB to the nucleus. We demonstrated that OxPAPC does not induce the NFκB signaling pathway in human umbilical vein endothelial cells [25] and HAEC [32]. In order to address a possible involvement of the NFκB pathway in endothelial activation by OxPAPC, we used different approaches: first, we demonstrated by Western blotting that OxPAPC, in contrast to TNF-α did not induce phosphorylation of IκBα. Furthermore, OxPAPC did not activate IκBα degradation, while TNF-α induced a rapid decrease in the levels of IκBα. Second, OxPAPC did not induce binding of p65 to its consensus DNA. Third, we have found that in contrast to LPS, OxPAPC did not stimulate a luciferase reporter construct bearing a 5xNFκB-consensus site. Fourth, overexpression of IκBα using adenovirus [33] had no influence on OxPAPC-induced endothelial activation, but significantly inhibited the effect of TNF-α. Altogether, these data demonstrate that induction of inflammation by OxPAPC is independent of the classical NFκB pathway in HUVEC [25].

3.4 Role of PKC and MAPK-dependent pathways in OxPL-induced monocyte adhesion

We [24] have shown previously that treatment with OxPAPC induced activation of both PKC and PKA. Recent data from our laboratory show that inhibition of PKC or PKA results in reduction of monocyte adhesion by OxPAPC or POVPC. We [25] have also shown that OxPAPC caused phosphorylation of ERK 1/2 within 20 min of stimulation which, in contrast to transient TNF-induced ERK activation, was sustained for up to 8 h. Activation of ERK 1/2 requires phosphorylation by its upstream kinase MEK 1/2, which can be activated by a PKC-dependent pathway. In addition, we found that OxPAPC induced phosphorylation of p38 MAP-kinase within 10 min of stimulation being sustained for 40 min. Blocking MEK1/2 as well as p38 using pharmacological inhibitors resulted in decreased OxPAPC-induced monocyte binding to HUVEC. Taken together, these data indicate that OxPL-induced activation of HUVEC leading to monocyte adhesion is dependent on protein kinase C/MEK/ERK, as well as p38-MAPK. Although the JNK pathway was also shown to be activated by OxPAPC [24], nothing is known about this pathway in OxPL-induced monocyte adhesion.

3.5 Role of $cPLA_2$ and lipoxygenase (LOX) in OxPL-induced monocyte adhesion

Among the targets that are directly phosphorylated and thereby activated by ERK and p38 MAPK is cytosolic phospholipase A_2 ($cPLA_2$) [34–36]. Thus, we asked whether $cPLA_2$ was involved in monocyte adhesion induced by OxPL. Pretreatment of HUVEC with the $cPLA_2$ inhibitor arachidonyltrifluoromethyl-ketone ($AACOCF_3$) for 3 h significantly reduced monocyte adhesion induced by both OxPAPC and POVPC (Huber *et al.*, unpublished observation). Moreover, treatment of HUVEC with OxPAPC increased levels of free AA in a time-dependent manner. The release of AA from phospholipids is the rate-limiting step in the synthesis of bioactive eicosanoids [37]. We [38] have shown previously that LOX inhibitors such as cinnamoyl-3,4-dihydroxy-a-cyanocinnamate (CDC) or 5,8,11,14-eicosatetraynoic acid (ETYA) blocked monocyte adhesion induced by OxPAPC. Metabolites generated by 12-LOX include 12(*S*)- and 12(*R*)-HETE. Treatment of HAEC with 12(*S*)-HETE but not 12(*R*)-HETE was shown to induce monocyte adhesion [39]. Accordingly, stimulation of HUVEC with 12(*S*)-HETE resulted in increased binding of monocytes in a concentration-dependent manner (Huber *et al.*, unpublished observation). These data indicate that OxPL-induced monocyte adhesion to HUVEC requires the release of AA by $cPLA_2$ and further modification by 12-LOX to generate 12(*S*)-HETE.

3.6 Can OxPL trigger vascular inflammation?

A large body of data obtained *in vitro* indicates that oxidized phospholipids can induce expression of cell adhesion molecules and chemokines in cells of the vascular wall, suggesting that lipid oxidation products can be regarded as triggers of the inflammatory response in atherosclerosis. Nevertheless, convincing proof of this concept *in vivo* is still missing.

Recently, we studied effects of OxPL by topically applying OxPAPC to murine carotid arteries using Pluronic gel as a vehicle, thereby mimicking their accumulation in the vessel wall during atherogenesis. The advantage of this model is that it allows for local application of OxPL, thus avoiding rapid uptake by liver and degradation of the substances by serum enzymes, both of which are observed during the systemic application of OxPL. Using this model we showed for the first time direct effects of OxPL on a blood vessel *in vivo*, clearly demonstrating that OxPAPC induced several chemokines after local application in the carotid artery [9]. In addition, we investigated OxPL-induced monocyte adhesion and rolling in *ex vivo* perfused carotid arteries [40]. We could show that KC (keratinocyte-derived chemokine, CXCL1), the murine chemokine related closest to human IL-8, and P-selectin play important roles in OxPAPC-induced monocyte adhesion and rolling to intact arteries [9].

3.6.1 Induction of chemokine expression by OxPAPC in murine carotid arteries *in vivo*

Chemokines serve a vital role in supporting the inflammatory response of the arterial wall leading to atherosclerotic plaque formation [41]. In particular, genetic deletions of MCP-1 or its receptor CCR2, as well as transplantation of bone marrow deficient in the IL-8 receptor homolog CXCR2 [42–44] have been shown to decrease monocyte accumulation and lesion formation in mice susceptible to atherosclerosis.

We investigated if OxPL induces expression of chemokines and monocyte adhesion in intact murine arteries. To model the accumulation of OxPL in the arterial wall, OxPAPC was topically applied to surgically exposed carotid arteries of C57BL/6 mice using pluronic F-127 gel as vehicle. F-127 dissolves within several hours and releases trapped lipids, allowing for topical exposure of arteries while minimizing systemic effects [9]. We used this system to investigate differential expression between LPS, OxPAPC, native PAPC, and mock-treated arteries of a set of chemokines, including MCP-1 and KC. Quantitative RT-PCR showed that treatment of carotid arteries for 6 h with 50 μg OxPAPC *in vivo* increased vascular expression of MCP-1 and KC, as compared to mock-treated arteries. In addition, treatment of carotid arteries with OxPAPC induced MIP-1α and MIP-1β, while RANTES, serum-derived factor-1 (SDF-1), and eotaxin were not induced [9]. Other genes that were implicated to play roles in atherosclerosis, such as EGR-1, tissue factor, IL-6, VEGF, and HO-1 were also up-regulated. Immunohistochemistry of carotid arteries treated for 24 h with OxPAPC demonstrated homogenous chemokine distribution throughout the vessel wall. Application of 50 μg nonoxidized PAPC to carotid arteries did not influence gene expression levels as compared to mock-treated arteries, demonstrating that oxidative modification of phospholipids was necessary to form proinflammatory agonists [9].

3.6.2 OxPAPC triggers monocyte rolling and arrest in native murine arteries mediated by P-selectin and KC

Chemokines support inflammation in atherogenesis by rapidly activating mononuclear leukocytes, leading to integrin-dependent cell arrest on inflamed endothelium, a prerequisite for transmigration [45]. To investigate if OxPAPC triggers rolling and firm adhesion of circulating monocytes on arterial endothelium, we used isolated carotid arteries from C57BL/6 mice. Arteries were perfused for 4 h with OxPAPC or native PAPC, and subsequently with calcein-labeled monocytic Mono-Mac-6 (MM6) cells [40]. Firm adhesion of MM6 cells was increased in OxPAPC-treated carotid arteries at the region of the bifurcation, whereas minimal adhesive interactions were observed in arteries treated with native PAPC [9]. Among the chemokines found to be up-regulated by OxPAPC in the artery wall, KC has been shown to play a dominant role in triggering monocyte arrest on early atherosclerotic endothelium in *ex vivo* perfused carotid arteries of *ApoE*$^{-/-P}$ mice [40]. We hypothesized that KC serves a similar function in OxPAPC-stimulated arteries. Indeed, preperfusion of a blocking KC antibody in OxPAPC-treated carotid arteries reduced MM6 cell arrest to levels seen in control arteries, indicating that OxPAPC-induced monocyte arrest was critically dependent on KC.

Functional blocking of P-selectin has been shown to abrogate monocyte rolling on atherosclerotic endothelium in isolated murine carotid arteries [46]. We found that preperfusion with a blocking P-selectin antibody abolished MM6 cell rolling in OxPAPC-treated arteries, indicating a crucial role for P-selectin in OxPAPC-triggered monocyte rolling.

Further analysis of chemokines that are induced by OxPL will help to establish a chemokine pattern that is responsible for monocyte specificity during "lipid-induced vascular inflammation." Possible interventional strategies emerging from such studies can also be tested for by applying Pluronic gel containing pharmacological substances, antisense oligonucleotides, or adenoviral vectors [47, 48] together with oxidized lipids to mouse arteries. As clinical trials to limit lipid oxidation in patients failed so far to beneficially influence vascular disease [49], more insight into molecular events triggered by oxidized lipids in the arterial wall might yield a clearer understanding of the pathophysiologic role of lipid oxidation in vascular disease as well as more promising targets for intervention.

4 Receptors involved in OxPL-induced inflammation

4.1 Involvement of a putative GPCR

There are indications that POVPC may act by binding to a membrane receptor (probably a G-protein coupled receptor [50]). In support of this hypothesis we have shown that the effects of POVPC and PEIPC can be blocked by PAF receptor antagonists [51]. It has been shown that OxPAPC also increases the level of cAMP in endothelial cells [22, 52]. Moreover, many bioactive phospholipids like lyso-PC, sphingosine-1-phosphate, and lysophosphatidic acid act *via* G-protein coupled receptors. In addition, our findings suggest that POVPC and PGPC act on different receptors, based on the different effects of these molecules [22].

4.2 A possible role for toll-like receptors (TLRs) in OxPL-induced inflammation

Although conflicting evidence exists about the role of bacteria in the development of cardiovascular disease, TLR4

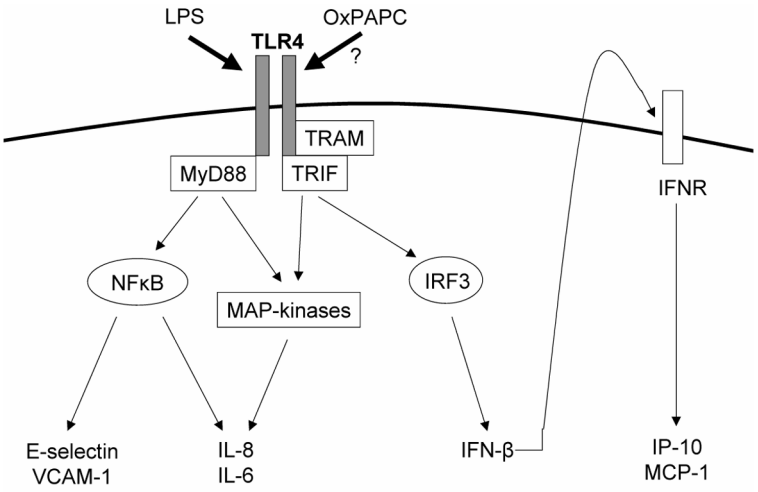

Figure 2. Major signaling pathways induced by LPS *via* TLR4.

has been recently implicated in atherogenesis [53]. Different studies demonstrated a protective role of a Asp299Gly-TLR4 polymorphism which, in carriers, seemed to result in an attenuation of atherosclerosis [54]. Endogenous TLR-ligands [55] have been implicated in the development of atherosclerosis. It is of note that the C3H/HeJ mouse strain, which carries a point mutation in the TLR4 gene, is largely resistant to atherosclerosis [56]. Interestingly endothelial cells, isolated from C3H/HeJ mice, show a reduced inflammatory response not only to lipopolysacharide (LPS), but also to oxidized lipids.

TLRs are an evolutionary highly conserved family of pattern recognition receptors (PRRs) that detect and bind pathogen-associated molecular patterns (PAMPs) such as LPS, peptidoglycans, lipoteichoic acid, or dsRNA [57]. So far, in mammals, 11 different TLRs have been identified, which bind distinct ligands. TLRs comprise a family of type I transmembrane receptors, which are characterized by an extracellular leucine-rich repeat domain and an intracellular Toll/IL-1 receptor (TIR) domain [58]. TLRs are expressed on various cell types, most prominently on cells involved in the regulation of inflammation and immunity such as macrophages, dendritic cells (DCs), or endothelial cells. Activation of TLRs is one initial event initiating host defense, regulating critical steps of the innate immune response, such as activation of endothelial cells and the following adhesion and transmigration of leukocytes. In addition TLRs trigger the maturation of DCs, which then eventually initiate an adaptive immune response [59].

LPS, a major component of the gram-negative bacterial cell wall, is one of the most potent exogenous activators of the innate and adaptive immune system. *In vivo*, bacteria-derived LPS is rapidly bound by the LPS binding protein (LBP) and then transferred to CD14, a protein that exists both in a soluble and in a GPI-anchored, membrane-bound, form. CD14 presents LPS to a complex consisting of TLR4 and MD2. Binding of LPS triggers homodimerization of TLR4 and association of several adaptor proteins to its intracellular TIR domain, a prerequisite for the activation of downstream signaling pathways [60]. MyD88 is one major TLR adaptor protein and is utilized by all so far identified TLRs, as well as by the interleukin-1 receptor. MyD88 triggers association and activation of protein kinases IRAK-1 and IRAK-4 (IL-1 receptor-associated kinase), which initiate a rapid activation of the transcription factor NFκB *via* activation of TRAF-6 (TNF-receptor-associated factor), and consequently induction of NFκB-dependent genes, such as TNF, E-selectin or interleukin 12 [61]. Studies [62, 63] in MyD88-deficient mice revealed the presence of a MyD88-independent pathway (Fig. 2), which is initiated *via* the alternative TLR-adaptor proteins TRAM and TRIF. TRIF facilitates activation of IRF3, a transcription factor which triggers production of interferon-β (IFN-β) (Fig. 2). Expression of IFN-β-inducible genes such as IP-10, as well as up-regulation of costimulatory molecules and MHC-complexes, are the consequence of this autocrine activation loop. In addition, TLR4 activates several other signaling pathways such as the MAP-kinase pathways, JNK, p38MAPK, and ERK 1/2. Different transcription factors including AP-1, Egr-1, or ATF-related transcription factors such as CREB are subsequently involved in downstream transcriptional events [61].

It was demonstrated that "MyD88-dependent signaling" may play an important role in atherosclerosis since MyD88 deficiency led to a reduction in plaque size and expression of proinflammatory genes [64, 65]. Moreover, TLR4 deficiency is associated with reduced atherosclerosis in apoE-deficient mice [66]. In contrast to TLR4, the role of TLR2 receptors in atherosclerosis has not been extensively studied. CD36, which has been shown to be a receptor for OxPL [67] may act in connection with TLR2 [68, 69].

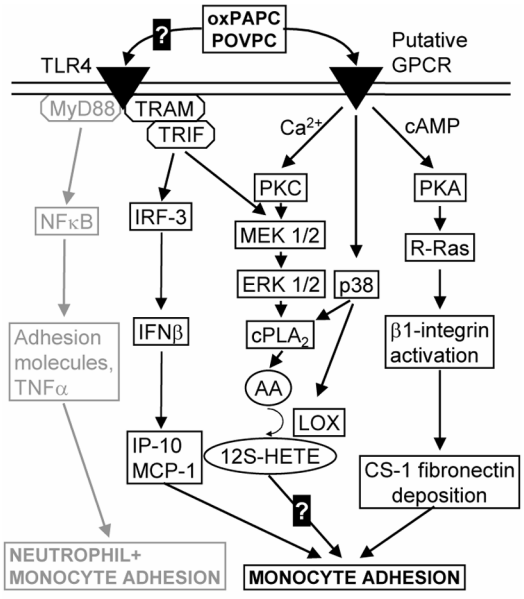

Figure 3. Signaling pathways induced by OxPL that lead to specific monocyte adhesion.

Evidence that OxPL resembles structural patterns that are recognized by PRRs comes from our studies demonstrating that OxPAPC inhibited LPS-induced inflammatory reactions by interacting with accessory plasma proteins CD14 and LPS-binding protein (LBP), which present LPS to its receptor TLR4 [70]. Others have shown that MM-LDL binds to CD14 [71], and OxPL binds to C-reactive protein [72]. Although the exact nature of a receptor for OxPAPC remains elusive, TLR4 itself has been recently implicated in OxPAPC-induced IL-8 synthesis [73] and macrophage activation [71]. It is of note that, although the gene expression pattern induced by OxPAPC and LPS overlaps, there are significant differences in terms of induced signaling cascades and induced gene expression [74]. LPS rapidly activates the transcription factor NFκB *via* the TLR-adaptor protein MyD88. In contrast to LPS, OxPAPC does not activate NFκB and does not induce genes like E-selectin or TNF, which are mainly NFκB-dependent. Genes induced by LPS as well as OxPAPC include MCP-1, IL-8, IL-6, and IP-10. Interestingly, TRIF is involved in the LPS-mediated induction of genes like MCP-1 and IP-10. A key step in this "MyD88-independent pathway" is the induction of IFN-β (Fig. 3). It has been shown that in MyD88 null endothelial cells monocyte adhesion induced by MM-LDL was significantly inhibited [64], indicating a role for the MyD88-dependent pathway in OxPL-induced monocyte adhesion. Strong indications for an involvement of TLR4 in OxPL-induced endothelial cell activation come from Judith Berliner's laboratory, showing that knocking down TLR4 using an antisense approach reduced OxPAPC-induced IL-8 expression [73]. Recently, Miller *et al.* [75] have shown that MM-LDL induced MIP-2 in mouse macrophages involving TLR4 and MyD88, while MCP-1 induction was independent of MyD88.

5 Conclusions and outlook

It has been shown previously that OxPL present in oxidized LDL and in atherosclerotic lesions specifically induce monocyte–endothelial interactions, an initiating event in the development of the atherosclerotic plaque. Activation of MAP-kinases rather than activation of the classical NF-κB pathway by oxidized lipids seems to mediate specific monocyte adhesion and expression of inflammatory genes in endothelial cells. Furthermore, stimulation of HUVEC by OxPL to bind monocytes involves activation of $cPLA_2$ and 12-LOX. The downstream effectors recruited by 12(*S*)-HETE that ultimately lead to enhanced monocyte adhesion might involve nuclear receptors that remain to be determined. Thus, multiple signaling pathways need to be activated for the induction of monocyte–endothelial interactions by OxPL (Fig. 3). Very little is known about receptors that mediate OxPL-induced effects. There are several indications that some of the OxPAPC components are recognized by a putative GPCR and evidence is accumulating for an involvement of TLRs (especially TLR4 and TLR2) in OxPL-induced endothelial activation.

Identification of signaling pathways, receptors, and chemokine expression patterns that are induced by OxPL and lead to specific monocyte adhesion are currently investigated in several laboratories. Results from these studies will provide new insights into the mechanisms by which monocytes are selectively recruited to chronically inflamed tissue. The molecular dissection of the upstream and downstream components of the vascular regulatory pathways induced by OxPL leading to specific monocyte adhesion and structural identification of individual biologically active lipids should ultimately lead to the development of novel therapeutic approaches against chronic inflammatory diseases. In addition, studies using mice deficient in TLR-signaling components should demonstrate whether OxPL utilize this pathway to induce vascular inflammation, and will help to identify individual components that can be regarded as culprits in this process. Together, characterizing the signaling pathways that lead to the monocyte-specific response induced by oxidized lipids as well as identifying genes and receptors that are involved in this mechanism may lead to new strategies of therapeutic intervention in the treatment of atherosclerosis and other chronic inflammatory diseases.

6 References

[1] Libby, P., *Nature* 2002, *420(6917)*, 868 – 874.

[2] Skalen, K., Gustafsson, M., Rydberg, E. K., Hulten, L. M., Wiklund, O., Innerarity, T. L., Boren, J., *Nature* 2002, *417(6890)*, 750 – 754.

[3] Lusis, A. J., *Nature* 2000, *407(6801)*, 233 – 241.

[4] Watson, A. D., Leitinger, N., Navab, M., Faull, K. F., Horkko, S., Witztum, J. L., Palinski, W. *et al.*, *J. Biol. Chem.* 1997, *272(21)*, 13597 – 13607.

[5] Subbanagounder, G., Watson, A. D., Berliner, J. A., *Free Radic. Biol. Med.* 2000, *28(12)*, 1751 – 1761.

[6] Berliner, J. A., Subbanagounder, G., Leitinger, N., Watson, A. D., Vora, D., *Trends Cardiovasc. Med.* 2001, *11(3 – 4)*, 142 – 147.

[7] Kadl, A., Huber, J., Gruber, F., Bochkov, V. N., Binder, B. R., Leitinger, N., *Vasc. Pharmacol.* 2002, *38(4)*, 219 – 227.

[8] Van Lenten, B. J., Wagner, A. C., Navab, M., Fogelman, A. M., *J. Biol. Chem.* 2001, *276(3)*, 1923 – 1929.

[9] Furnkranz, A., Schober, A., Bochkov, V. N., Bashtrykov, P., Kronke, G., Kadl, A., Binder, B. R. *et al.*, *Arterioscler. Thromb. Vasc. Biol.* 2005, *25(3)*, 633 – 638.

[10] Halliwell, B., *Free Radicals in Biology and Medicine*, Clarendon Press, Oxford 1989.

[11] Zhang, R., Brennan, M. L., Shen, Z., MacPherson, J. C., Schmitt, D., Molenda, C. E., Hazen, S. L., *J. Biol. Chem.* 2002, *277(48)*, 46116 – 46122.

[12] Esterbauer, H., Jurgens, G., Quehenberger, O., Koller, E., *J. Lipid Res.* 1987, *28(5)*, 495 – 509.

[13] Podrez, E. A., Poliakov, E., Shen, Z., Zhang, R., Deng, Y., Sun, M., Finton, P. J. *et al.*, *J. Biol. Chem.* 2002, *277(41)*, 38503 – 38516.

[14] Berliner, J. A., Territo, M. C., Sevanian, A., Ramin, S., Kim, J. A., Bamshad, B., Esterson, M., Fogelman, A. M., *J. Clin. Invest.* 1990, *85(4)*, 1260 – 1266.

[15] Berliner, J., Leitinger, N., Watson, A., Huber, J., Fogelman, A., Navab, M., *Thromb. Haemost.* 1997, *78(1)*, 195 – 199.

[16] Huber, J., Vales, A., Mitulovic, G., Blumer, M., Schmid, R., Witztum, J. L., Binder, B. R., Leitinger, N., *Arterioscler. Thromb. Vasc. Biol.* 2002, *22(1)*, 101 – 107.

[17] Arroyo, A., Modriansky, M., Serinkan, F. B., Bello, R. I., Matsura, T., Jiang, J., Tyurin, V. A. *et al.*, *J. Biol. Chem.* 2002, *277(51)*, 49965 – 49975.

[18] Subbanagounder, G., Leitinger, N., Schwenke, D. C., Wong, J. W., Lee, H., Rizza, C., Watson, A. D. *et al.*, *Arterioscler. Thromb. Vasc. Biol.* 2000, *20(10)*, 2248 – 2254.

[19] Chang, M. K., Bergmark, C., Laurila, A., Horkko, S., Han, K. H., Friedman, P., Dennis, E. A., Witztum, J. L., *Proc. Natl. Acad. Sci. USA* 1999, *96(11)*, 6353 – 6358.

[20] Chang, M. K., Binder, C. J., Miller, Y. I., Subbanagounder, G., Silverman, G. J., Berliner, J. A., Witztum, J. L., *J. Exp. Med.* 2004, *200(11)*, 1359 – 1370.

[21] Shih, P. T., Elices, M. J., Fang, Z. T., Ugarova, T. P., Strahl, D., Territo, M. C., Frank, J. S. *et al.*, *J. Clin. Invest.* 1999, *103(5)*, 613 – 625.

[22] Leitinger, N., Tyner, T. R., Oslund, L., Rizza, C., Subbanagounder, G., Lee, H., Shih, P. T. *et al.*, *Proc. Natl. Acad. Sci. USA* 1999, *96(21)*, 12010 – 12015.

[23] Cole, A. L., Subbanagounder, G., Mukhopadhyay, S., Berliner, J. A., Vora, D. K., *Arterioscler. Thromb. Vasc. Biol.* 2003, *23(8)*, 1384 – 1390.

[24] Birukov, K. G., Leitinger, N., Bochkov, V. N., Garcia, J. G., *Microvasc. Res.* 2004, *67(1)*, 18 – 28.

[25] Bochkov, V. N., Mechtcheriakova, D., Lucerna, M., Huber, J., Malli, R., Graier, W. F., Hofer, E. *et al.*, *Blood* 2002, *99(1)*, 199 – 206.

[26] Reddy, S., Hama, S., Grijalva, V., Hassan, K., Mottahedeh, R., Hough, G., Wadleigh, D. J. *et al.*, *J. Biol. Chem.* 2001, *276(20)*, 17030 – 17035.

[27] Kronke, G., Bochkov, V. N., Huber, J., Gruber, F., Bluml, S., Furnkranz, A., Kadl, A. *et al.*, *J. Biol. Chem.* 2003, *278(51)*, 51006 – 51014.

[28] Yeh, M., Gharavi, N. M., Choi, J., Hsieh, X., Reed, E., Mouillesseaux, K. P., Cole, A. L. *et al.*, *J. Biol. Chem.* 2004, *279(29)*, 30175 – 30181.

[29] Yeh, M., Cole, A. L., Choi, J., Liu, Y., Tulchinsky, D., Qiao, J. H., Fishbein, M. C. *et al.*, *Circ. Res.* 2004, *95(8)*, 780 – 788.

[30] Davies, S. S., Pontsler, A. V., Marathe, G. K., Harrison, K. A., Murphy, R. C., Hinshaw, J. C., Prestwich, G. D. *et al.*, *J. Biol. Chem.* 2001, *276(19)*, 16015 – 16023.

[31] Mercurio, F., Manning, A. M., *Curr. Opin. Cell Biol.* 1999, *11(2)*, 226 – 232.

[32] Yeh, M., Leitinger, N., de Martin, R., Onai, N., Matsushima, K., Vora, D. K., Berliner, J. A., Reddy, S. T., *Arterioscler. Thromb. Vasc. Biol.* 2001, *21(10)*, 1585 – 1591.

[33] Wrighton, C. J., Hofer-Warbinek, R., Moll, T., Eytner, R., Bach, F. H., de Martin, R., *J. Exp. Med.* 1996, *183(3)*, 1013 – 1022.

[34] Sa, G., Murugesan, G., Jaye, M., Ivashchenko, Y., Fox, P. L., *J. Biol. Chem.* 1995, *270(5)*, 2360 – 2366.

[35] Kramer, R. M., Roberts, E. F., Um, S. L., Borsch-Haubold, A. G., Watson, S. P., Fisher, M. J., Jakubowski, J. A., *J. Biol. Chem.* 1996, *271(44)*, 27723 – 27729.

[36] Schmidlin, F., Loeffler, S., Bertrand, C., Landry, Y., Gies, J. P., *Naunyn Schmiedebergs Arch. Pharmacol.* 2000, *361(3)*, 247 – 254.

[37] Smith, W. L., *Am. J. Physiol.* 1992, *263(2 Pt 2)*, F181 – F191.

[38] Honda, H. M., Leitinger, N., Frankel, M., Goldhaber, J. I., Natarajan, R., Nadler, J. L., Weiss, J. N., Berliner, J. A., *Arterioscler. Thromb. Vasc. Biol.* 1999, *19(3)*, 680 – 686.

[39] Patricia, M. K., Kim, J. A., Harper, C. M., Shih, P. T., Berliner, J. A., Natarajan, R., Nadler, J. L., Hedrick, C. C., *Arterioscler. Thromb. Vasc. Biol.* 1999, *19(11)*, 2615 – 2622.

[40] Huo, Y., Weber, C., Forlow, S. B., Sperandio, M., Thatte, J., Mack, M., Jung, S. *et al.*, *J. Clin. Invest.* 2001, *108(9)*, 1307 – 1314.

[41] Weber, C., Schober, A., Zernecke, A., *Arterioscler. Thromb. Vasc. Biol.* 2004, *24(11)*, 1997 – 2008.

[42] Gu, L., Okada, Y., Clinton, S. K., Gerard, C., Sukhova, G. K., Libby, P., Rollins, B. J., *Mol. Cell* 1998, *2(2)*, 275 – 281.

[43] Boring, L., Gosling, J., Cleary, M., Charo, I. F., *Nature* 1998, *394(6696)*, 894 – 897.

[44] Boisvert, W. A., Santiago, R., Curtiss, L. K., Terkeltaub, R. A., *J. Clin. Invest.* 1998, *101(2)*, 353 – 363.

[45] Weber, C., *J. Mol. Med.* 2003, *81(1)*, 4 – 19.

[46] Ramos, C. L., Huo, Y., Jung, U., Ghosh, S., Manka, D. R., Sarembock, I. J., Ley, K., *Circ. Res.* 1999, *84(11)*, 1237 – 1244.

[47] Hu, Y., Zou, Y., Dietrich, H., Wick, G., Xu, Q., *Circulation* 1999, *100(8)*, 861 – 868.

[48] Calvo, W. J., Hajduczok, G., Russell, J. A., Diamond, S. L., *Circulation* 1999, *99(8)*, 1069 – 1076.

[49] Steinberg, D., Witztum, J. L., *Circulation* 2002, *105(17)*, 2107 – 2111.

[50] Parhami, F., Fang, Z. T., Yang, B., Fogelman, A. M., Berliner, J. A., *Arterioscler. Thromb. Vasc. Biol.* 1995, *15(11)*, 2019 – 2024.

[51] Subbanagounder, G., Leitinger, N., Shih, P. T., Faull, K. F., Berliner, J. A., *Circ. Res.* 1999, *85(4)*, 311 – 318.

[52] Parhami, F., Fang, Z. T., Yang, B., Fogelman, A. M., Berliner, J. A., *Arterioscler. Thromb. Vasc. Biol.* 1995, *15(11)*, 2019 – 2024.

[53] Vink, A., Schoneveld, A. H., van der Meer, J. J., van Middelaar, B. J., Sluijter, J. P., Smeets, M. B., Quax, P. H. *et al.*, *Circulation* 2002, *106(15)*, 1985 – 1990.

[54] Kiechl, S., Lorenz, E., Reindl, M., Wiedermann, C. J., Oberhollenzer, F., Bonora, E., Willeit, J., Schwartz, D. A., *N. Engl. J. Med.* 2002, *347(3)*, 185 – 192.

[55] Beg, A. A., *Trends Immunol.* 2002, *23(11)*, 509 – 512.

[56] Shi, W., Wang, N. J., Shih, D. M., Sun, V. Z., Wang, X., Lusis, A. J., *Circ. Res.* 2000, *86(10)*, 1078 – 1084.

[57] Medzhitov, R., *Nature Rev. Immunol.* 2001, *1(2)*, 135 – 145.

[58] Takeda, K., Akira, S., *Semin. Immunol.* 2004, *16(1)*, 3 – 9.

[59] Akira, S., Takeda, K., Kaisho, T., *Nat. Immunol.* 2001, *2(8)*, 675 – 680.

[60] Akira, S., Yamamoto, M., Takeda, K., *Biochem. Soc. Trans.* 2003, *31(Pt 3)*, 637 – 642.

[61] Akira, S., Hoshino, K., *J. Infect. Dis.* 2003, *187 (Suppl 2)*, S356 – S363.

[62] Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O. *et al.*, *Science* 2003, *301(5633)*, 640 – 643.

[63] Beutler, B., *Curr. Opin. Immunol.* 2000, *12(1)*, 20 – 26.

[64] Michelsen, K. S., Wong, M. H., Shah, P. K., Zhang, W., Yano, J., Doherty, T. M., Akira, S. *et al.*, *Proc. Natl. Acad. Sci. USA* 2004, *101(29)*, 10679 – 10684.

[65] Bjorkbacka, H., Kunjathoor, V. V., Moore, K. J., Koehn, S., Ordija, C. M., Lee, M. A., Means, T. *et al.*, *Nat. Med.* 2004, *10(4)*, 416 – 421.

[66] Michelsen, K. S., Doherty, T. M., Shah, P. K., Arditi, M., *Circ. Res.* 2004, *95(12)*, e96 – e97.

[67] Podrez, E. A., Poliakov, E., Shen, Z., Zhang, R., Deng, Y., Sun, M., Finton, P. J. *et al.*, *J. Biol. Chem.* 2002, *277(41)*, 38517 – 38523.

[68] Hoebe, K., Georgel, P., Rutschmann, S., Du, X., Mudd, S., Crozat, K., Sovath, S. *et al.*, *Nature* 2005, *433(7025)*, 523 – 527.

[69] Tobias, P., Curtiss, L. K., *J. Lipid Res.* 2005, *46(3)*, 404 – 411.

[70] Bochkov, V. N., Kadl, A., Huber, J., Gruber, F., Binder, B. R., Leitinger, N., *Nature* 2002, *419(6902)*, 77 – 81.

[71] Miller, Y. I., Viriyakosol, S., Binder, C. J., Feramisco, J. R., Kirkland, T. N., Witztum, J. L., *J. Biol. Chem.* 2003, *278(3)*, 1561 – 1568.

[72] Chang, M. K., Binder, C. J., Torzewski, M., Witztum, J. L., *Proc. Natl. Acad. Sci. USA* 2002, *99(20)*, 13043 – 13048.

[73] Walton, K. A., Hsieh, X., Gharavi, N., Wang, S., Wang, G., Yeh, M., Cole, A. L., Berliner, J. A., *J. Biol. Chem.* 2003, *278(32)*, 29661 – 29666.

[74] Leitinger, N., *Curr. Opin. Lipidol.* 2003, *14(5)*, 421 – 430.

[75] Miller, Y. I., Viriyakosol, S., Worrall, D. S., Boullier, A., Butler, S., Witztum, J. L., *Arterioscler. Thromb. Vasc. Biol.* 2005, *25(6)*, 1213 – 1219.

