The role of oxidized phospholipids in atherosclerosis.

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Summary

Atherosclerotic cardiovascular disease (ASCVD) is the most prevalent cause of death in the Western world. The most important risk factor for ASCVD is elevated plasma low-density lipoprotein (LDL)-cholesterol levels. Oxidation of LDL leads to the formation of ox-LDL, which is taken up by the cells of the arterial wall. Elevated plasma levels of oxidized LDL (ox-LDL) are associated with CVD. Ox-LDL contains high levels of oxidized phospholipids (oxPL). OxPL itself is highly pro-inflammatory and pro-atherogenic in mice. In humans, oxPL also circulates on lipoprotein (a) [Lp(a)], which is also hypothesized to be pro-atherogenic. Lp(a) plasma levels are elevated in ~20% of all humans and are associated with increased ASCVD. This has been attributed to the oxPL circulating on Lp(a). This review will focus on the role of oxPL, and thus both ox-LDL and Lp(a), in ASCVD-related morbidity and mortality.

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Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the most prevalent cause of death in the Western world. It is estimated that approximately 500 million people suffer from ASCVD and that ASCVD causes about 19 million deaths yearly [1]. Furthermore, the cost per ASCVD patient is over US$20.000 per patient [2]. ASCVD is a lipid-driven chronic inflammatory disease of the arteries.

Risk factors for ASCVD are, among others, elevated plasma low-density lipoprotein (LDL)-cholesterol, smoking, obesity, diabetes, sedentary lifestyle, and high blood pressure. Statins that lower LDL-cholesterol are the most commonly used medication for ASCVD. Despite statins decreasing ASCVD risk by ~30%, a large residual ASCVD risk remains [3].

This indicates that there is a need for the development of alternative treatments to improve ASCVD outcomes. LDL is a crucial lipoprotein that transports hydrophobic lipids such as cholesterol and fatty acids. Elevated plasma LDL-cholesterol is a causal risk factor for ASCVD [4]. After modification, such as oxidation, LDL-cholesterol is taken up by cells of the arterial wall.

A recent analysis of various studies carried out between 2000 and 2022 has shown that elevated plasma levels of oxidized LDL (ox-LDL) are associated with CVD, especially in patients with chronic inflammation [5]. The chronic inflammation was reflected by high levels of pro-inflammatory cytokines, including Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), and IL-1β.

Ox-LDL contains high levels of oxidized phospholipids (oxPL). OxPL itself is highly pro-inflammatory and pro-atherogenic in mice [6, 7]. In humans, oxPL also circulates on lipoprotein (a) [Lp(a)], a small lipoprotein particle that contains apolipoprotein B100 (apoB100) and apolipoprotein (a) [apo(a)], and that is pro-atherogenic [7]. I will focus on the role of oxPL, and thus both ox-LDL and Lp(a), in ASCVD-related morbidity and mortality.

Atherosclerosis

The onset of atherosclerosis is characterized by endothelial dysfunction; a marker for early atherosclerosis. High levels of circulating ox-LDL may cause endothelial dysfunction via impairment of the nitric oxide (NO) production, which leads to elevated Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation that upregulates expression of adhesion molecules [8]. Increased expression of adhesion molecules on endothelial cells (ECs) leads to the infiltration of monocytes that, once present in the

arterial wall, differentiate into macrophages that take up ox-LDL via Cluster of differentiation 36 (CD36), leading to foam cell formation [9]. In addition, ox-LDL or oxPL triggers inflammatory responses by acting on Toll-like receptors (TLRs) on the surface of macrophages [10]. In later stages of atherosclerosis, macrophages secrete various factors including Transforming growth factor-β (TGF-β) and Interleukin-1β (IL-1β) that stimulate smooth muscle cell (SMC) proliferation and migration to the cap of the plaque. Here, SMCs produce collagen, contributing to plaque stability [11]. When macrophages in the plaque become highly pro-inflammatory, they secrete matrix metalloproteinases (MMPs), such as MMP2 and MMP9 that degrade collagen, leading to cap thinning, and subsequent plaque rupture [12]. This can lead to thrombus formation. Thrombi can cause serious harm by occluding already narrowed arteries as such preventing the blood flow which provides oxygen to vital organs including the heart and brain. This causes clinical complications such as heart attack or stroke.

Oxidation of LDL

Several factors may contribute to LDL oxidation, including oxidative stress and inflammation accompanied by releasing reactive oxygen species (ROS) or free oxygen radicals [13, 14]. For example, endothelial cells may release ROS upon oxidative stress, which induces LDL modifications. As a consequence, LDL uptake by macrophages occurs 3 to 4 times more rapidly in comparison to regular LDL that is not modified by endothelial cells [15]. Factors such as SOD (superoxide dismutase) and CAT (catalase) inhibit LDL oxidation and thus decrease the uptake of ox-LDL by macrophages [16]. Once taken up by macrophages, ox-LDL may induce cell death, reflected by the release of lactate dehydrogenase (LDH) [17], or may induce inflammation, as further described below.

OxPL, Ox-LDL, and inflammation

In endothelial cells, ox-LDL can be recognized by TLR4, a main receptor involved in inflammation. This induces activation of TLR4's adaptor protein myeloid differentiation primary response protein 88 (MyD88) and downstream activation of the transcription factor (TF) Nuclear factor kappa B (NF-κB) and Activator Protein 1 (AP-1), resulting in the production of IL-8 and C-C motif chemokine ligand 2 (CCL2), also known as Monocyte chemoattractant protein-1 (MCP-1). IL-8 is a chemokine that attracts and activates neutrophils. MCP-1 induces monocyte adhesion to the endothelium, and subsequent infiltration of monocytes into plaques in a process known as diapedesis [18]. Monocytes subsequently differentiate into macrophages. Ox-LDL incubation thus induces monocyte and neutrophil adhesion to endothelial cells.

In macrophages, ox-LDL is recognized by the scavenger receptor CD36. CD36 can recognize various patterns associated with microbes [10]. It also recognizes phosphocholine-rich (PC) lipoteichoic acid (LTA) secreted by Gram-positive bacteria [19]. PC is also a key component of oxPL; this leads to CD36-mediated interactions without the presence of exogenous microbes as depicted in *Figure 1*. CD36 can interact with TLR4 [20]. TLR4 usually forms homodimers. However, when interacting with ox-LDL, TLR4 forms a heterodimer with TLR6 [21].

Upon internalization of ox-LDL via CD36, The novel Lck/yes-related protein tyrosine kinase (Lyn) is activated, which subsequently phosphorylates Tyrosine-residues on the Toll/Interleukin-1 Receptor (TIR) domains of TLR4 and TLR6 [20]. Phosphorylation allows for the formation of the TLR4-TLR6 heterodimer and subsequent interaction with MyD88, which is an adaptor protein that binds to the TIR domains of TLR4 and TLR6 and regulates downstream signaling [20].

In addition to forming a complex with TLR4-TLR6, CD36 also forms a complex with TLR2 [21]. TLR2 cannot form homodimers but needs to heterodimerize to exert its effects. When ox-LDL is recognized by CD36, CD36 forms a complex with TLR2 and TLR2 forms a heterodimer with TLR6, leading to the formation of the CD36-TLR2-TLR6 complex. Similar to the CD36-TLR4-TLR6 complex, the CD36-TLR2-TLR6 complex interacts with MyD88 to regulate downstream signaling [21].

First, MyD88 is recruited to the CD36-TLR4-TLR6 or CD36-TLR2-TLR6 complex. Once the recruitment of MyD88 is completed, MyD88 facilitates the binding of several serine/threonine IL-1R-associated kinases (IRAKs). MyD88 and IRAKs form the Myddosome complex [22]. The Myddosome complex can subsequently recruit and activate the TNF receptor-associated factor 6 (TRAF6). TRAF6 mediates the activation of Transforming growth factor beta-activated kinase 1 (TAK1). TAK1 has a crucial role in inducing NF-κB signaling. TAK1 phosphorylates and thereby activates the IκB kinase (IKK) complex [23]. Upon activation of the IKK complex, this complex phosphorylates the inhibitor of NF-κB (IκB). The phosphorylation of IκB induces its degradation [23]. Degradation of this complex leads to the subsequent liberation of NF-κB; a p65 (RelA) and p50 heterodimer. This activation of NF-κB allows for its translocation into the nucleus where it binds DNA [23]. Upon binding DNA it induces transcription of certain genes, specifically those which encode inflammatory chemokines and cytokines, including pro-IL-1β and pro-IL-18 [4]. These observations thus indicate a model where ox-LDL binding to the CD36-TLR4-TLR6 or CD36-TLR2-TLR6 complex facilitates the induction of pro-inflammatory gene expression, including pro-IL-1β and pro-IL-18.

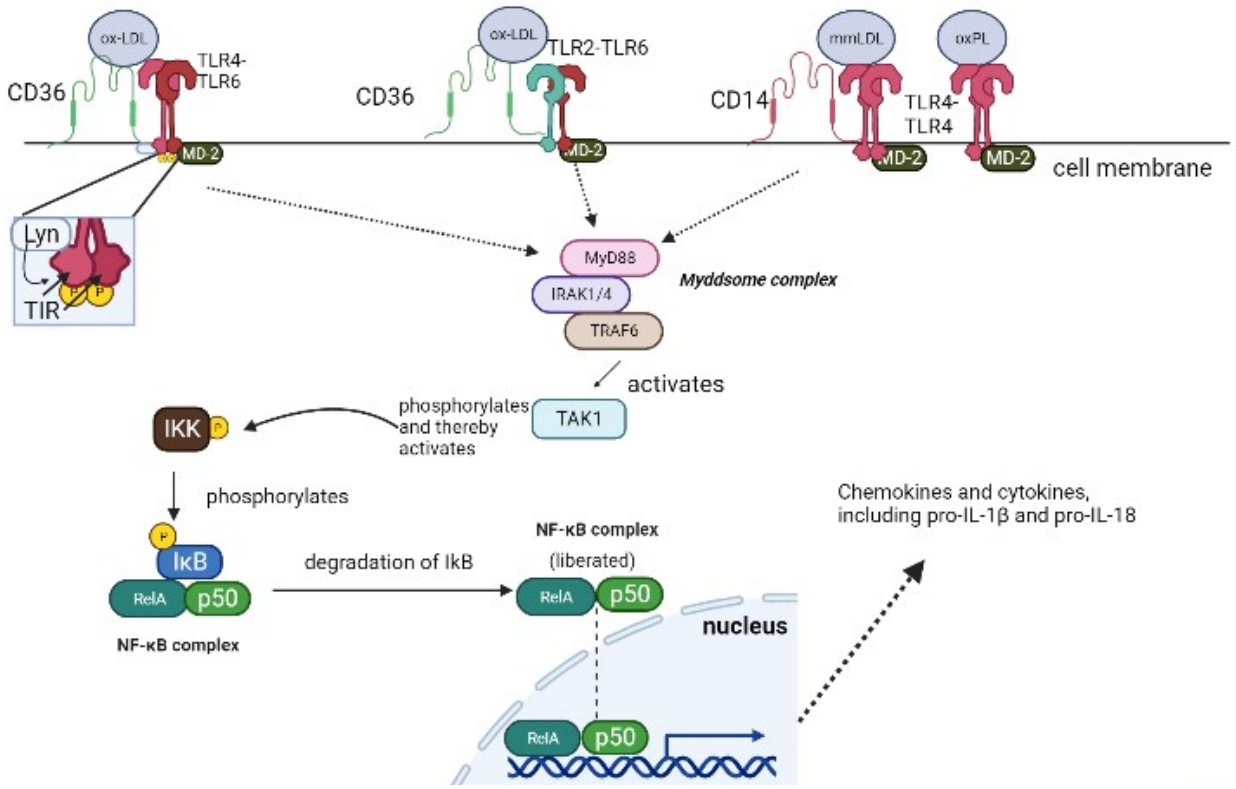

Figure 1: The recognition of oxidized low-density lipoprotein (ox-LDL) mediates the formation of the Scavenger receptor Cluster of differentiation (CD36) - Toll-like receptor 4 - Toll-like receptor 6 (CD36-TLR4-TLR6) or the CD36-TLR2-TLR6 complex. Upon recognition of ox-LDL via CD36, the novel Lck/yes-related protein tyrosine kinase (Lyn) is activated This leads to the phosphorylation of Tyrosine-residues on the Toll/Interleukin-1 Receptor (TIR) domains of TLR4 and TLR6. This induces dimer formation. Furthermore, mmLDL (minimally modified LDL) interacts with TLR4, mediated by CD14, and thereby contributes to inflammation. Myeloid differentiation factor 2 (MD-2) binds TLR4 and forms a complex. CD36-TLR2-TLR6 and CD36-TLR4-TLR6 also contain an MD-2 intracellularly. These complexes recruit (MyD88). Upon this recruitment, IRAK1 (Interleukin-1 Receptor-Associated Kinase 1) and IRAK4 (Interleukin-1 Receptor-Associated Kinase 4) are bound to myeloid differentiation primary response protein 88 (MyD88) and form the Myddsome complex. This complex recruits and activates TRAF6 (TNF Receptor-Associated Factor 6). Upon activation, TRAF6 can activate TAK1 (Transforming growth factor beta-activated kinase 1). TAK1 phosphorylates and thereby activates the IKK (IκB Kinase) complex. Subsequently, phosphorylated IKK can phosphorylate IκB. Phosphorylated IκB is subsequently degraded, which induces the liberation of NF-κB (Nuclear Factor-Kappa B), consisting of v-rel avian reticuloendotheliosis viral oncogene homolog A (RelA) and p50. Liberated NF-κB can now enter the nucleus and bind DNA. Here, it induces the transcription of multiple target genes which encode inflammatory chemokines and cytokines, including pro-Interleukin-1β (pro-IL-1β) and pro-IL-18.

As stated above, oxPL is a main component of ox-LDL. Interestingly, a polymorphism in IL-1 potentiates the cardiovascular risk in patients with high levels of apolipoprotein B100-bound oxPL, suggesting an interaction between oxPL and IL-1 in cardiovascular disease [24]. The Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial has shown that IL-1β-induced inflammation enhances myocardial infarction in humans [25]. A seminal study demonstrated that ox-LDL may induce the secretion of IL-1β by macrophages [26], suggesting that elevated ox-LDL levels in patients with myocardial infarction may contribute to elevated inflammation. The secretion of IL-1β by macrophages is regulated by inflammasomes, of which the NOD-like receptor protein 3 (NLRP3) inflammasome is the most broadly studied. How ox-LDL may activate the NLRP3 inflammasome is depicted in *Figure 2* and further described below.

For the NLRP3 inflammasome to be activated, it needs two signals, a priming signal, and an activation signal. The priming signal induces the activation of NF-κB and the transcription of the various components of the NLRP3 inflammasome complex, including NLRP3, Apoptosis-associated speck-like protein containing a CARD (ASC), and pro-caspase-1 [27]. In the case of ox-LDL, binding to the CD36-TLR4-TLR6 or CD36-TLR2-TLR6 complex may function as an NLRP3 inflammasome priming step. Next, an activation step is needed, leading to the assembly of the NLRP3 inflammasome and subsequent ASC-mediated caspase-1 activation, resulting in the cleavage of pro-caspase-1 into its active form. Thereafter, active caspase-1 cleaves pro-IL-1β and pro-IL-18 into IL-1β and IL-18, eventually leading to their secretion [28].

Ox-LDL does not only act as an NLRP3 inflammasome priming signal but can also act as a second signal for NLRP3 inflammasome activation. Upon internalization of ox-LDL into the macrophage via the Scavenger receptor CD36, the vesicle containing the ox-LDL undergoes fusion with the lysosome. The lysosome hydrolyzes ox-LDL into free cholesterol, resulting in lysosomal free cholesterol accumulation. The lysosome typically has a low pH, but when free cholesterol accumulates, the pH inside the lysosome increases. The increase in pH disrupts the membrane integrity of the lysosome. This leads to the efflux of K^+ [28]. K^+ efflux may lead to the activation of the NLRP3 inflammasome. Furthermore, cholesterol crystals can also induce inflammasome activation. Upon ox-LDL internalization, free cholesterol can accumulate in the macrophage, eventually leading to the crystallization of cholesterol. The presence of cholesterol crystals can induce lysosomal damage by destabilizing and disrupting the lysosome [28]. Nevertheless, the validity of this theory remains debated, since not all studies found accumulation of cholesterol crystals when cholesterol accumulates in lysosomes [29]. The induced lysosomal damage acts as a signal for the inflammasome to subsequently cleave pro-caspase-1 into caspase-1. This active form now cleaves pro-IL-1β and pro-IL-18 into active IL-1β and IL-18 [30].

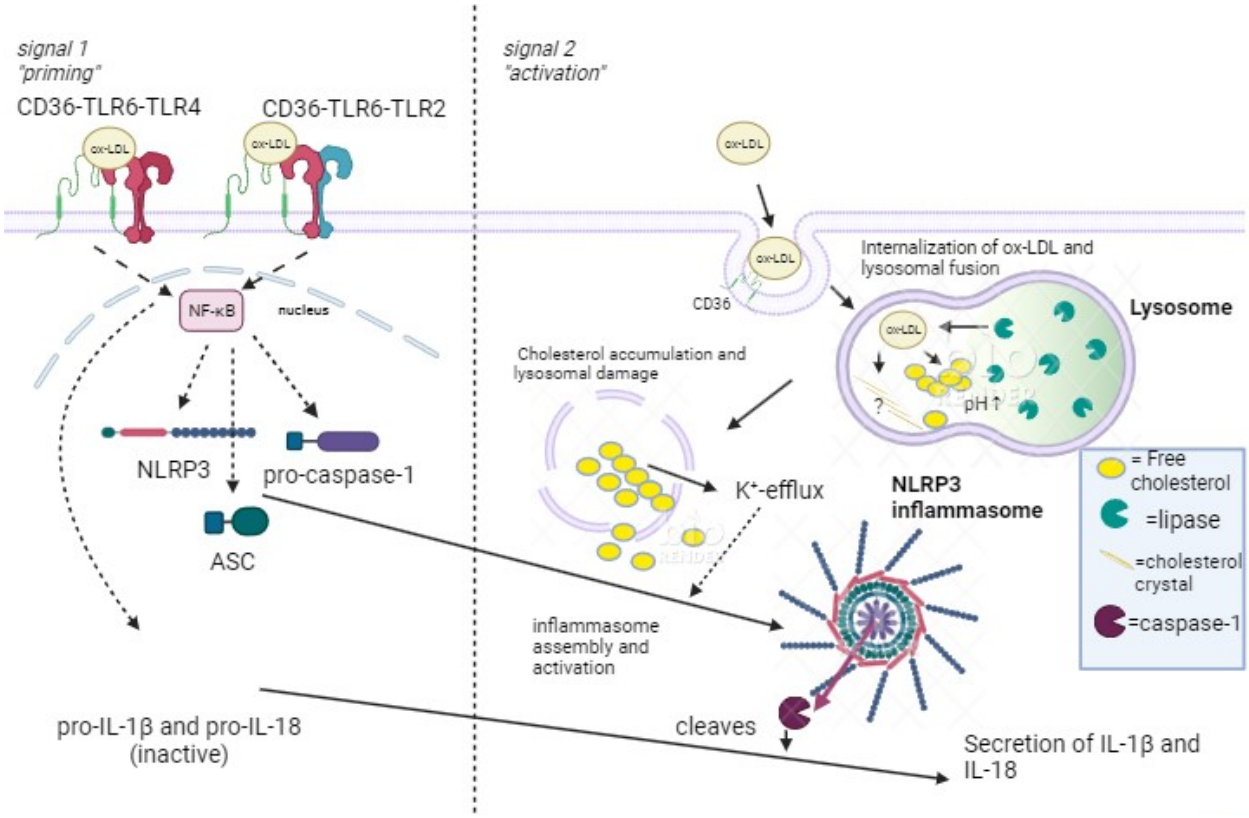

Figure 2: *For the production of Interleukin (IL)-1β and IL-18, two signals are required: signals 1 and 2, priming and activation respectively. Signal 1 is mediated by Oxidized low-density lipoprotein (ox-LDL). Ox-LDL can be recognized by the Scavenger receptor Cluster of differentiation (CD36) - Toll-like receptor 4 - Toll-like receptor 6 (CD36-TLR4-TLR6) and the CD36-TLR2-TLR6 complexes. This recognition leads to the subsequent activation of the Nuclear Factor Kappa B (NF-κB), NF-κB is responsible for the production of NOD-like receptor family pyrin domain-containing 3 (NLRP3), Apoptosis-associated speck-like protein containing a CARD (ASC), pro-caspase-1, pro-IL-1β, and pro-IL-18. Signal 2 is also triggered by ox-LDL. Upon recognition of ox-LDL by CD36, ox-LDL is internalized via vesicle formation. This vesicle subsequently fuses with the lysosome. Here the ox-LDL is hydrolyzed and subsequently broken down into free cholesterol. This degradation of ox-LDL leads to the accumulation of cholesterol in the lysosome. This accumulation leads to an elevated pH which results in lysosomal damage and rupture. Also, free cholesterol accumulation may induce the crystallization of free cholesterol, leading to the formation of cholesterol crystals. These crystals may then also lead to lysosomal damage and subsequent rupture. Lysosomal damage and rupture result in the efflux of K^+-ions. Lysosomal damage leads to the assembly of the NLRP3-inflammasome and the subsequent activation. In the inflammasome, pro-caspase-1 is converted into caspase-1, which can subsequently cleave the previously NF-κB mediated production of inactive pro-IL-1β, and pro-IL-18 into active IL-1β and IL-18.*

Several studies have shown that TLR activation and NLRP3 activation aggravate atherosclerosis. In apoE$^{-/-}$ mice, deficiency of CD36 in bone marrow cells significantly reduces the burden of atherosclerotic lesions even at the late stages of the disease [31], which has been attributed to a decrease in ox-LDL uptake. In both human and mouse studies, TLR2 and TLR4 expression was elevated in atherosclerotic lesions [32,33]. Furthermore, TLR4 or TLR2 deficiency reduces atherosclerosis in LDLr$^{-/-}$ mice [34,35]. In addition, deficiency of MyD88 which is downstream of TLR2 and TLR4, decreases atherosclerotic lesion size, as shown by Björkbacka and colleagues [36]. These findings suggest a crucial role for TLR-mediated signaling in atherosclerosis.

NLRP3 is highly expressed in aortic samples of atherosclerotic patients and is positively correlated with disease severity [37]. A deficiency of NLRP3 or its adaptor protein decreases atherosclerosis in Ldlr$^{-/-}$ mice [38].

In addition to ox-LDL, minimally modified low-density lipoprotein (mmLDL) interacts with TLR4 mediated by CD14, inducing the production of pro-inflammatory cytokines and ROS by macrophages [39], but mmLDL does not lead to NLRP3 inflammasome activation [40].

In summary, ox-LDL induces NLRP3 inflammasome activation, mediated by both effects on priming and activation. As a result, IL-1β secretion is increased. Interestingly, antibodies to IL-1β decrease recurrent cardiovascular events in humans as shown in the CANTOS trial [25]. Together, these data suggest that ox-LDL-induced NLRP3 inflammasome activation may be an important contributor to CVD in humans.

Lipoprotein(a) and ASCVD

Ox-LDL exerts various of its pro-inflammatory effects via oxPL. OxPL directly induces inflammation by activating TLR4. In humans, up to ~85% of oxPL in plasma may be bound to Lp(a) [41]. Lp(a) plasma levels are elevated in ~20% of all humans [42]. Lp(a) is associated with increased ASCVD, as shown by genome-wide association studies (GWAS) and Mendelian randomization studies [43, 44]. This has been attributed to the oxPL circulating on Lp(a). Therefore, I will further focus on the role of Lp(a) in ASCVD.

Lp(a) consists of apoB100, apo(a), and lipids including oxPL. Apo(a) has multiple Kringle IV repeats [45]. The number of repeats is genetically determined and regulated by the *LPA* gene. 91% of the variations in plasma Lp(a) concentrations are accounted for by the apo(a) gene [46]. In this gene, the number of Kringle IV repeats accounted for 69% of the variation in plasma levels [46]. In humans with a smaller number of repeats, and thus a small Lp(a) isoform size, plasma Lp(a) levels are elevated and cardiovascular risk is increased [47].

Pro-atherogenic effects of Lp(a)-bound oxPL

OxPL is bound to the KIV_{10} domain of apo(a) by the strong lysine-binding site (LBS). Apo(a) variants lacking this KIV_{10} domain show no oxPL binding [48]. Lp(a) is usually not expressed by mice. However, when expressed in mice, it was shown that mice transgenic for the variant of apo(a) that lacked the LBS in KIV_{10} were less susceptible to atherosclerosis compared to transgenic mice expressing apo(a) without this mutation [49], suggesting a critical role for oxPL in effects of Lp(a) on atherosclerosis.

Several laboratories have investigated how Lp(a) exerts its pro-inflammatory and pro-atherogenic effects. Early studies found that Lp(a) induces the adhesion of monocytes to human endothelial cells [50, 51]. Later, it was shown that Lp(a) induces the production of IL-6 by monocytes [52]. In addition, Lp(a) enhances IL-8 secretion from macrophages [51]. Interestingly, this effect was inhibited when apo(a) lacking the LBS was used, suggesting that this effect was dependent on the binding of oxPL to apo(a).

Employing the E06 antibody that binds most oxPL, several studies have shown that oxPL is pro-inflammatory and pro-atherogenic. Mice expressing E06 showed a reduction in inflammation and atherosclerosis [7]. This was mainly attributed to oxPL on ox-LDL and apoptotic cells since mice do not naturally express apo(a) [53]. Moreover, *in vitro* studies have shown that E06 can inhibit the pro-inflammatory effects of Lp(a) in endothelial cells, monocytes, and macrophages [61, 50, 7].

In endothelial cells, Lp(a) increases the expression of various adhesion molecules such as Endothelial-selectin (E-selectin), Intercellular Adhesion Molecule 1 (ICAM-1), and Vascular Cell Adhesion Molecule 1 (VCAM-1) [54, 55], which are critical for monocyte adhesion. These effects were diminished by co-incubation with E06, indicating that these effects were dependent on Lp(a) bound oxPL.

The question arises as to the mechanism of the effects of Lp(a) on endothelial cells. It has been suggested that oxPL bound to apo(a) interferes with the endothelial cell barrier function. Mechanistically, this is mediated by effects of apo(a) bound oxPL on 1) glycolytic flux; and 2) Rho (the Ras homologous)/ROCK (Rho-kinase)-dependent signaling.

Lp(a) and glycolytic flux

By increasing the glycolytic flux via the transcription of glycolytic enzymes and Glucose Transporter 1 (GLUT1) production, apo(a)-bound oxPL induces the transcription of pro-inflammatory genes that mediate monocyte adhesion, including VCAM-1 and ICAM-1,

or chemokines including MCP-1 that also promotes monocyte adhesion [56]. Basically, energy is required for these processes, and Lp(a) upregulates glycolysis to generate an energy source to induce inflammation.

When plaques accumulate within the arterial wall, this can induce arterial narrowing that may lead to hypoxia, as oxygen-rich blood cannot be transported to the tissue. Under hypoxic circumstances, anaerobic metabolism takes place. This leads to the production of lactate. This eventually leads to the amplification of glycolytic output and thereby upregulation of ATP production [57]. This ATP production is essential to generate energy to sustain inflammation Enhancing the glycolytic output results in the production of ROS [60]. ROS activates NF-κB. The Lp(a) induced increase in glycolytic flux is accompanied by the production of MCP-1, ICAM-1, VCAM-1, IL-6, and IL-8 [58]. When apo(a) without the LBS was used, this effect was inhibited [58]. This indicates that the oxPL bound to apo(a) was necessary for it to take place. Essentially, via increasing glycolytic flux, Lp(a) activates NF-κB. Increasing NF-κB signaling enhances the expression of adhesion molecules and thereby promotes vascular permeability.

Lp(a) not only enhances permeability by inducing the production of adhesion molecules; it also impairs endothelial barrier function via stress fiber formation.

Lp(a) and Rho/ROCK mediated stress fiber formation

Apo(a) triggers the activation of Rho via the activation of guanine nucleotide exchange factor (GEF) [61]. The downstream signaling pathway is depicted in *Figure 3* . Normally, Rho is in its inactive state by binding to GDP. The activation of Rho is mediated by GEF. GEF catalyzes the conversion of Rho-GDP into Rho-GTP, the active form of Rho. Active Rho-GTP binds the Rho-binding domain (RBD) of ROCK. In the inactive state, ROCK is inhibited with the interaction of the pleckstrin homology (PH)-domain and the RBD [65]. Both the PH-domain and the RBD can bind the amino-terminal kinase region. This interaction auto-inhibits ROCK [65]. However, when Rho-GTP binds this inhibits the interaction between the PH-domain, RBD, and the kinase region and thereby activates ROCK. Activation of ROCK enables it to exert kinase activity [65]. Upon activation, ROCK mediates the phosphorylation of the myosin phosphatase target subunit 1 (MYPT1) [61]. MYPT1 is the regulatory subunit of MLC phosphatase (MLC-P). MLC-P induces the inactivation of the myosin light chain (MLC) by dephosphorylating it [62]. However, when MLC-P is inhibited it cannot exert its dephosphorylation ability [66]. Subsequently, MLC cannot be dephosphorylated and thus remains in its active state. Persistent MLC activity leads to stress fiber formation [66]. The formation of these fibers makes the endothelial cells contract and lose their shape. Altering the cell structure increases the permeability of the endothelial monolayer, thereby impairing EC barrier function. However, when an LBS-deficient variant of apo(a) was used and thus lacking oxPL, the effects of apo(a) was

diminished, again indicating that the effects of Lp(a) on inducing endothelial permeability were dependent on apo (a) bound oxPL [65]. Thus, oxPL bound to Lp(a) is also crucial for inducing its effects on vascular permeability changes by altering cell shape.

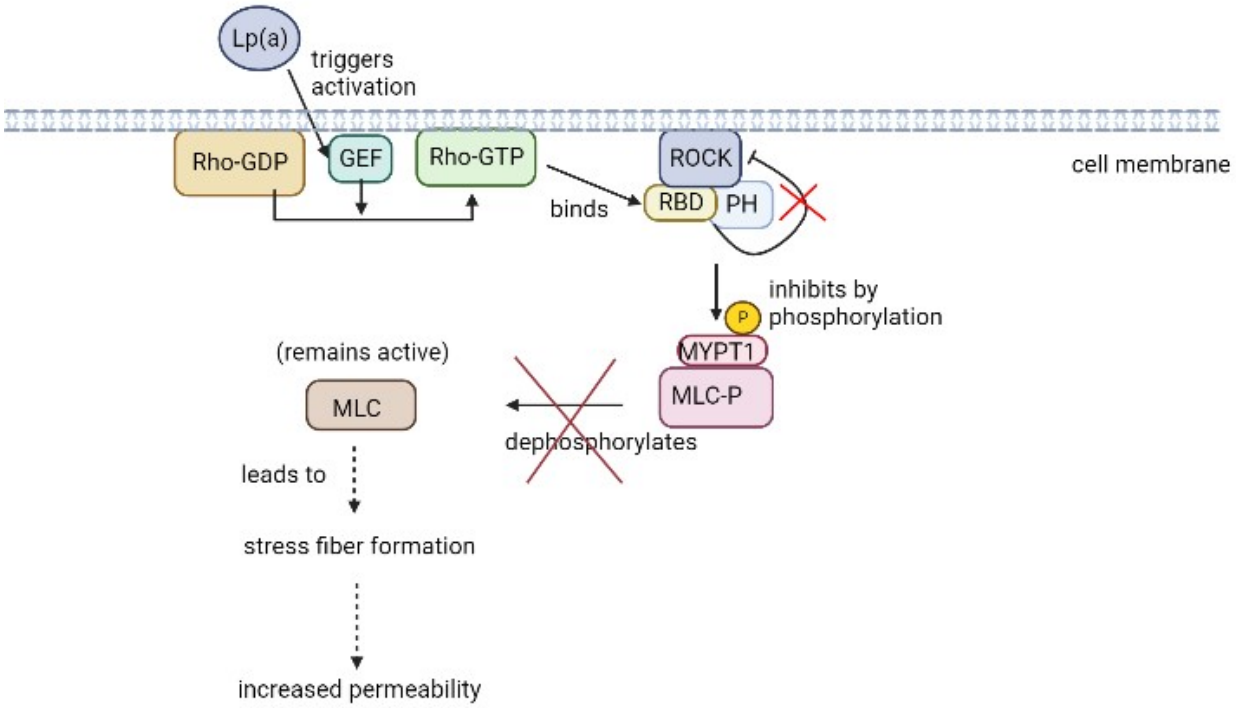

Figure 3: Lp(a) triggers the activation of GEF (Guanine nucleotide exchange factor). GEF can convert inactive Rho-GDP (Rho guanosine diphosphate) into active Rho-GTP (Rho guanosine triphosphate). Active Rho-GTP binds to the RBD (Rho-binding domain) of ROCK (Rho-associated coiled-coil kinase) and thereby inhibits the autoinhibitory effect of the RBD and PH (pleckstrin homology) domain. Activated ROCK can now phosphorylate the MYPT1 (myosin phosphatase target subunit 1) domain of MLC-P (myosin light chain phosphatase). MYPT1 is the regulatory subunit of MLC-P. Normally, MLC-P dephosphorylates MLC (myosin light chain) and thereby inactivates it. However, by inhibiting MLC-P, MLC cannot be dephosphorylated. This results in a persistent active state of MLC. Active MLC mediates the formation of stress fibers. Stress fiber formation leads to cell contraction and thereby leads to increased permeability of the endothelial layer.

Downstream of Rho/ROCK signaling, Lp(a) triggers the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) mediated signaling pathway [63]. This is depicted in

Figure 4. PI3K can phosphorylate phosphoinositides such as 4,5-bisphosphate (PIP2) [67]. Phosphorylating PIP2 converts it into phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 can now recruit Akt, and together PIP3 and Akt form a complex. Subsequently, Akt is phosphorylated by phosphoinositide-dependent kinase 1 (PDK-1) and mechanistic target of rapamycin complex 2 (mTORC2) [68]. Phosphorylation of Akt makes it active and thus able to phosphorylate GSK3β [67]. GSK3β normally phosphorylates β-catenin. However, when GSK3β is phosphorylated by Akt it is inactivated and it thus cannot phosphorylate β-catenin [67]. Phosphorylating β-catenin marks it for degradation. When β-catenin cannot be phosphorylated, its subsequent degradation will be inhibited. Prevention of degradation will result in the stabilization of β-catenin, allowing for its accumulation. Stabilizing β-catenin allows for its translocation into the nucleus, where it can function as a transcriptional coactivator [67]**.** Upon translocation, β-catenin can form a complex with various transcription factors allowing the complex to activate gene transcription. B-catenin induced transcriptional activation leads to the upregulation of the expression of Cyclooxygenase-2 (COX-2) [67]. COX-2 can convert arachidonic acid into prostaglandin H2 (PGH2). PGH2 can then be converted into, among others, PGE2. PGE2 leads up to increased vascular permeability [67]. Also here, apo(a) lacking the LBS did not exert any effect on COX-2 expression [67]. This again indicates that oxPL bound to apo(a) is required to ultimately induce COX-2 expression and thereby increase the permeability of the endothelial layer.

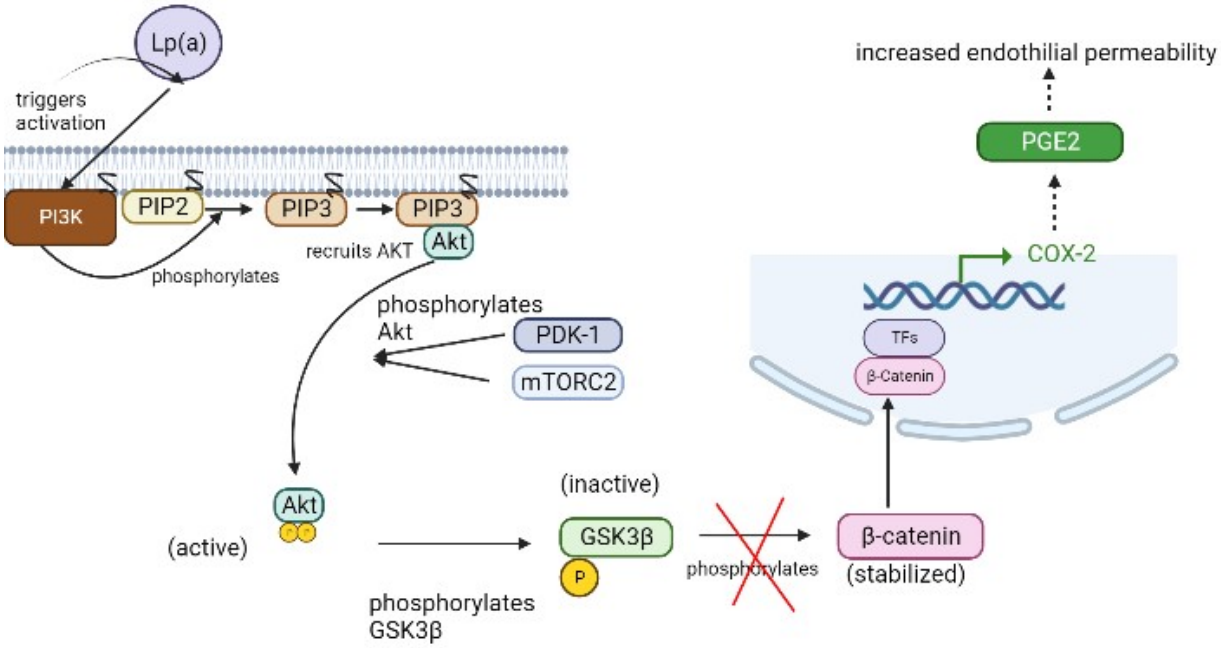

Figure 4: Lp(a) triggers PI3K (Phosphoinositide 3-kinase) activation. Activated PI3K can convert PIP2 (Phosphatidylinositol 4,5-bisphosphate) into PIP3 (Phosphatidylinositol 3,4,5-trisphosphate). Subsequently, PIP3 can recruit Akt (Protein kinase B) and form a complex. PDK-1 (Phosphoinositide-dependent kinase-1) and mTORC2 (mammalian target of rapamycin complex 2) can both phosphorylate Akt. Phosphorylation activates Akt, and active Akt can phosphorylate GSK3β (Glycogen synthase kinase 3 beta). Normally, GSK3β phosphorylates β-catenin. However, when GSK3β is inactive, it cannot phosphorylate β-catenin. Phosphorylation of β-catenin induces its subsequent degradation. However, when this does not occur, it can stabilize and thereby accumulate. This accumulation allows for the translation of β-catenin. Together with various Transcription Factors (TFs), it can bind DNA and induce the transcription of the COX-2 (Cyclooxygenase-2) gene, which eventually leads to the production of PGE2 (Prostaglandin E2). PGE2 increases the permeability of the endothelial layer.

Discussion

Oxidized LDL and oxidized phospholipids bound to ApoB100 or Apo(a) are associated with an increased incidence of CVD in humans [5, 49]. I here describe via which mechanisms ox-LDL or other modified forms of LDL, including mmLDL, and oxPL are pro-atherogenic. While oxPL and mmLDL directly act on the TLR4 to exert their pro-inflammatory and pro-atherogenic effects, for ox-LDL a variety of mechanisms has been described that all involve the binding of ox-LDL to its receptor CD36. While oxPL circulates on ox-LDL, the majority (~85%) of oxPL circulates on Lp(a) [41]. OxPL on Lp(a) enhances inflammation [61]. In addition to oxPL signaling mediated by TLR4, a series of mechanisms have been described that mediate Lp(a) induced inflammation. These mechanisms are still mainly mediated by oxPL and entail signaling pathways downstream of TLR4 signaling.

Interestingly, for ox-LDL to be pro-inflammatory, a complex of its receptor CD36 with TRL4 and TLR6 or TLR2 and TLR6 needs to be formed. The ox-LDL will first bind CD36 and then activate NF-κB signaling as soon as CD36 interacts with these Toll-like receptors [20, 21]. Ox-LDL does not need to be internalized for these effects. This step also induces NLRP3 inflammasome priming. However, to activate the NLRP3 inflammasome, internalization of ox-LDL via CD36 is required, leading to the accumulation of free cholesterol in the lysosome. This process promotes NLRP3 inflammasome assembly, caspase-1 cleavage, and secretion of IL-1β and IL-18 [26]. IL-1β is a master regulator of inflammation. IL-1β directly acts on its receptor on endothelial cells/myeloid cells, leading to NF-κB activation and increased expression of inflammatory genes [70]. The CANTOS trial has clearly suggested that IL-1β signaling enhances cardiovascular events in humans [26]. The outcome of the CANTOS trial and the finding that ox-LDL increases NLRP3 inflammasome activation and

IL-1β secretion suggests that patients with elevated plasma ox-LDL may benefit from anti-inflammatory medications, such for instance NLRP3 inflammasome inhibitors.

Of interest, an IL-1 polymorphism that promotes inflammation together with elevated oxPL, which mainly circulates on Lp(a), was associated with increased ASCVD in humans [24]. This suggests another interaction between inflammatory pathways and lipid-related pathways in promoting inflammation and ASCVD. Several studies have shown that oxPL accounts for the pro-inflammatory effects of Lp(a). Perhaps a certain level of low-grade inflammation is amplified by these Lp(a) bound oxPL resulting in deleterious effects. Medications to lower oxPL have so far not been proven to be effective [69], but this has mainly been attributed to technical issues; these medications did not really lower circulating oxPL levels. Therapies to lower apo(a) levels are currently in development and will likely concomitantly lower plasma oxPL, and thus, inflammation. In sum, several pathways mediate the pro-inflammatory effects of certain lipids or lipoproteins in ASCVD. While therapies may be targeted at lowering oxPL or ox-LDL or the lipoproteins they circulate on directly, when not effective, additional approaches could involve decreasing inflammation, especially the inflammatory pathways that ox-LDL and oxPL act on.

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Afterword

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