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# Thrombogenic and Atherogenic Activities of Lysophosphatidic Acid

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**Abstract** Lysophosphatidic acid (LPA) has been identified as a biologically active lipid in mildly-oxidized LDL, human atherosclerotic lesions, and the supernatant of activated platelets. The evidence that LPA has thrombogenic and atherogenic activities has increased substantially in recent years. Supporting the thrombogenic activity of LPA, analysis of the core region of human carotid plaques revealed recently the presence of alkyl- and acyl-molecular species from LPA with high platelet-activating potency (16:0 alkyl-LPA, 20:4 acyl-LPA). LPA, lipid extracts of atherosclerotic plaques, and the lipid-rich core elicited shape change and, in synergy with other platelet stimuli, aggregation of isolated platelets. This effect was completely abrogated by prior incubation of platelets with LPA receptor antagonists. Furthermore, LPA at concentrations approaching those found in vivo, induced platelet shape change, aggregation, and platelet-monocyte aggregate formation in blood. LPA-stimulated platelet aggregation was mediated by the ADP-stimulated activation of the $P2Y_1$ and $P2Y_{12}$ receptors. Supporting its atherogenic activity, LPA is a mitogen and motogen to vascular smooth muscle cells (VSMCs) and an activator of endothelial cells and macrophages. Recently, LPA has been identified as an agonist of the peroxisome proliferator activating receptor $\gamma$ (PPAR$\gamma$), which is a key regulator of atherogenesis. LPA elicits progressive neointima formation, which is fully abolished by GW9662, an antagonist of PPAR$\gamma$. We propose that LPA plays a central role in eliciting vascular remodeling and atherogenesis. Furthermore, upon rupture of lipid-rich atherosclerotic plaques, LPA may trigger platelet aggregation and intra-arterial thrombus formation. Antagonists of LPA receptors might be useful in preventing LPA-elicited thrombus formation and neointima formation in patients with cardiovascular diseases. J. Cell. Biochem. 92: 1086–1094, 2004. 

**Key words:** lysophosphatidic acid; LPA receptors; platelet; thrombus; endothelial cells; vascular smooth muscle cells; monocytes; macrophages; PPAR-gamma; neointima

Oxidative modifications of LDL and platelet activation are central events in the pathogenesis of atherosclerosis and cardiovascular disease. Lipid-rich atheromatous plaques containing oxidized lipids and LDL are vulnerable and upon rupture this material comes into contact with circulating platelets, activates them and causes the formation of an intravascular platelet- and fibrin-rich thrombus that could lead to myocardial infarction and ischemic stroke.

In this context we have previously shown: (1) that mildly-oxidized LDL (mox-LDL) but not native LDL stimulates platelets and induces neointima formation, (2) that lysophosphatidic acid (LPA) is formed during mild oxidation of LDL, and (3) that LPA is the component of the mox-LDL- particle that mediates platelet shape change and endothelial cell contraction because LPA receptor antagonists fully block these cell responses, (4) activated platelets secrete phospholipases that provide a de novo pool of lysophospholipids that are substrates of lysophospholipase D-mediated LPA production in plasma, whereas lipid phosphate phosphatases decrease plasma LPA content [Weidtmann et al., 1995; Siess et al., 1999; Aoki et al., 2002; Sano et al., 2002; Smyth et al., 2003]. We further have shown that (5) platelet-activating LPA accumulates in the intima of atherosclerotic plaques that were removed by surgery from human femoral and carotid arteries as well as from the aorta, and that (6) the LPA content within the atherosclerotic lesion of carotid artery is highest in the lipid-rich core

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that consists mainly of extracellular lipid droplets and beds of foam cells [Siess et al., 1999].

In addition to its possible thrombogenic activities, LPA has potent atherogenic activities. LPA or LPA associated with the mox-LDL particle may play a role in the early phase of atherogenesis by stimulating endothelial cell contraction and the expression of adhesion molecules, thus increasing endothelial permeability and monocyte adhesion to the endothelium [Rizza et al., 1999; Siess et al., 1999; Essler et al., 2000; Siess, 2002]. Moreover, LPA is a mitogen and motogen for vascular smooth muscle cells (VSMCs), and stimulates their contraction thereby increasing in many animal models the vascular tone [Tigyi et al., 1995; Tigyi, 2001; Siess, 2002]. Furthermore, we reported recently, that human monocytes, macrophages, and the monocytic cell line Mono Mac 6 express transcripts encoding $LPA_1$ and $LPA_2$ receptors [Fueller et al., 2003]. In Mono Mac 6 cells, which express transcripts for the $LPA_3$ receptor, LPA induced $Ca^{2+}$ transients with an $EC_{50}$ of 47 nM that was partially inhibited by dioctyl glycerol pyrophosphate (DGPP 8:0) and pertussis toxin (PTX). In these cells, mox LDL and serum also elicited $Ca^{2+}$ transients that cross desensitized with LPA and were partially inhibited by *N*-palmitoyl serine phosphoric acid (NPSerPA). In human monocytes or macrophages, the $Ca^{2+}$ response to LPA was highly variable (Zahler, Fueller and Siess, unpublished observations). For this reason, responses in human monocytes and macrophages elicited by LPA remain unclear. The activation of peroxisome proliferator activating receptor $\gamma$ (PPAR$\gamma$) and subsequent activation of the CD36 scavenger receptor in macrophages is an exciting possibility (see below) [McIntyre et al., 2003].

The possible athero- and thrombogenic actions of LPA have been described and discussed in an in-depth review previously and will not be duplicated here [Siess, 2002]. This brief study will focus on the very recent and exciting developments that give strong support to the notion of LPA being a thrombo- and atherogenic molecule.

## Working Hypothesis and Questions

In order to address the hypothesis that LPA is an important thrombogenic and atherogenic lipid accumulating in atherosclerotic lesions, we asked the following questions:

- Which LPA molecular species are generated during platelet activation, and LDL oxidation?
- Which LPA molecules are present in atherosclerotic lesions?
- What is the relative potency of LPA molecular species to activate platelets?
- What responses do mox-LDL and LPA elicit on the intact, non-injured vascular bed?
- What is the relative potency of LPA molecular species to induce neointima formation?
- Which receptors mediate LPA-induced platelet activation and neointima formation?
- Which signal transduction pathways are activated by LPA in platelets?
- Can the lipid-rich core of atherosclerotic lesions able to directly activate platelets? If yes, what is the contribution of LPA?
- Does LPA activate platelets in whole blood? If yes, through what mechanism?

## LPA Molecular Species in Platelet Supernatant, Plasma, and Serum

Stable-isotope-dilution electrospray-ionization liquid chromatography mass spectrometry (SIDEI-LC-MS) was applied to quantify the different molecular species of LPA generated in the supernatant of thrombin-stimulated platelets and also in clotting whole blood collected from healthy human volunteers. The plasma concentration of acyl-LPA was found to be in the high nanomolar range ($\sim$100 nM) [Saulnier-Blache et al., 2000; Sano et al., 2002]. Platelets stimulated with thrombin in the presence of EDTA, which prevents their aggregation and also the normal function of $Ca^{2+}$-dependent enzymes, did not generate LPA. In contrast, under these same conditions platelets rapidly secrete sphingosine-1-phosphate (S1P) into the medium [Yatomi et al., 2000; Sano et al., 2002]. In the presence of physiological concentrations of $Ca^{2+}$, thrombin-stimulated platelets generated LPA that was dominated by 18:2 and 20:4 species (20 and 34% of total LPA, respectively) [Gerrard and Robinson, 1989; Sano et al., 2002]. However, the total amount of LPA derived from isolated platelets remains only a fraction of that generated ex vivo during blood clotting [Aoki et al., 2002; Sano et al., 2002]. The serum concentration of LPA is in the 5–10 $\mu$M range

[Saulnier-Blache et al., 2000; Baker et al., 2001; Aoki et al., 2002; Sano et al., 2002]. In serum, the molecular species of LPA profile are again dominated by the polyunsaturated species, which implies that lysophospholipid precursors of LPA originate from phospholipase $A_1$ cleavage of the saturated fatty acids abundant in the *sn*-1 position on the glycerol backbone. Because arachidonate and linoleate are predominantly in the *sn*-2 position of plasma phospholipids, hence the observation that these two fatty acyl species dominate LPA in serum implies three distinct steps in their production which include: (1) $PLA_1$ cleavage, (2) acyl migration from *sn*-2 to *sn*-1 position, and (3) lysophospholipase D cleavage of the headgroup. The enrichment of polyunsaturated LPA species carries important physiological consequences. First, 20:4 LPA is more potent in activating platelets than other saturated or mono-unsaturated acyl-species [Tokumura et al., 2002; Rother et al., 2003]. Second, 20:4 and 18:2 LPA are the most potent acyl species that activate neointima formation and PPARγ, whereas the saturated acyl species are inactive [Yoshida et al., 2003; Zhang et al., 2003]. Thus, activation of platelets not only increases the concentration of LPA but also changes its acyl-chain composition in such a way that it is enriched in thrombogenic and atherogenic species.

### Identification of LPA Molecular Species With High Platelet-Activating Potency in the Lipid-Rich Core of Human Atherosclerotic Plaques

Atherosclerotic tissue specimens were obtained from patients who underwent operations for high-grade carotid stenosis. The carotid plaque tissue was removed by a technique of intraoperative endarterectomy that preserved the plaque structure en bloc [Brandl et al., 1997]. The histopathology of carotid endarterectomy specimens shows marked differences in the various regions, which represent different stages of the atherosclerotic process [Brandl et al., 1997]. The lipid-rich core is characterized histologically by extracellular lipid deposits and beds of foam cells. Based on previous results that this region had the highest LPA content, atherosclerotic specimens containing soft, lipid-rich plaques were collected. The lipid-rich core region was carefully dissected and homogenized, and LPA molecular species were quantified using SIDEI LC-MS. This analysis showed the presence of acyl-LPA (80%) and alkyl-LPA (20%) species in the lipid-rich core regions. The dominating acyl-LPA species was LPA (18:0), the relative amounts of LPA (18:1), LPA (16:0), and LPA (20:4) were less. In contrast, the predominant alkyl-LPA species was LPA (18:1); LPA (18:0) and LPA (16:0) were less abundant. The biological LPA titer of these samples as estimated by platelet shape change bioassay were higher than those determined by LC-MS, which might be explained by a different platelet-activating potency of the various LPA molecules present in the lipid-rich core [Rother et al., 2003].

Indeed, by comparing the biological effect of three molecular species of LPA found in the lipid-rich core, we found that alkyl-LPA (16:0) ($EC_{50}$ 0.9 nM) and acyl-LPA (20:4) ($EC_{50}$ 2.5 nM) were about 20- and 7-fold more potent at inducing platelet shape change than acyl-LPA (16:0). Therefore, these LPA species, although present in lesser amounts, due to their higher potency might be particularly important for platelet-activation elicited by the lipid rich core [Rother et al., 2003].

### Platelet LPA Receptors and Signaling Pathways

RT-PCR analysis of platelets revealed the presence of transcripts for all three EDG-family LPA receptors [Motohashi et al., 2000]. $LPA_4$ mRNA could not be detected in a megakaryocytic cell line suggesting that platelets do not express this receptor [Noguchi et al., 2003]. The LPA responses of platelets are not consistent with the pharmacological properties of the EDG family receptors $LPA_{1-3}$. Alkyl-LPA 16:0 is at least 20-times more potent in activating platelets than its acyl counterpart [Simon et al., 1982; Rother et al., 2003] and is the preferred ligand of PPARγ [Zhang et al., 2003, 2004]. Cyclic-phosphatidic acid (cPA) and NPSerPA, both agonists of the three EDG family LPA receptors yet are agonists as well as antagonists on platelets [Sugiura et al., 1994; An et al., 1998; Siess et al., 1999; Rother et al., 2003]. Furthermore, DGPP 8:0, an antagonist of LPA-receptors of the EDG-family with preference to $LPA_3$ over $LPA_1$ without an effect on $LPA_2$ receptors, inhibits LPA-induced platelet activation [Fischer et al., 2001; Rother et al., 2003]. DGPP 8:0 and PA 8:0 were the only PA and LPA analogs that lacked agonistic activity on isolated platelets. Whether all these LPA receptor antagonists exert their effect on platelets exclusively through the EDG family LPA receptors or in

addition through an elusive platelet LPA receptor remains to be elucidated. In support of the heterogeneity of LPA receptors in platelets, Tokumura et al. [2002] noted that while platelets collected from all donors responded to acyl LPA 18:1, some of them did not respond to alkyl-LPA 16:0. In this study, the authors found that platelets from a donor with platelets non-responsive to alkyl-LPA expressed mRNA to $LPA_2 > LPA_3 > LPA_1$ receptors. DGPP 8:0 and NPSerPA inhibited both acyl-LPA and alkyl-LPA responses [Rother et al., 2003]. Thus, the possibility remains that LPA responses in platelets are mediated via yet unidentified receptor(s).

What is known about the signaling pathways activated by LPA in platelets? Low nanomolar concentrations of LPA and mox-LDL induce shape change of washed platelets through LPA receptor linked signal transduction pathways that involve the activation of the heterotrimeric $G_{12}/G_{13}$ protein, the small GTPase Rho and Rho-kinase, and the Rho-kinase-mediated inhibition of myosin light chain phosphatase and stimulation of myosin light chain phosphorylation [Bauer et al., 1999; Retzer and Essler, 2000; Retzer et al., 2000]. This pathway mediates the reorganization of the actin cytoskeleton underlying platelet shape change. In addition, LPA and mox-LDL stimulate a different pathway during shape change, i.e., the activation of the Src-family of tyrosine kinases and the tyrosine kinase Syk, which might mediate the exposure of fibrinogen-binding sites on the integrin$\alpha$II$\beta$3, which is a prerequisite for platelet aggregation [Maschberger et al., 2000; Bauer et al., 2001].

High micromolar concentrations of LPA are needed to stimulate in platelets $Ca^{2+}$-entry and weak $Ca^{2+}$-mobilisation from intracellular stores, presumably through the activation of $G_q$ [Siess et al., 1999; Maschberger et al., 2000]. LPA does not activate the heterotrimeric G-protein $G_i$ in platelets, yet it shows a strong synergism with platelet stimuli that activate $G_i$ (epinephrine, ADP) in inducing platelet aggregation [Haserück et al., 2003; Rother et al., 2003].

### The Lipid-Rich Core Induces Platelet Shape Change and Triggers Platelet Aggregation Through the Activation of LPA Receptors

We observed that the lipid-rich core, as well as lipid extracts from the lipid-rich core, was capable of inducing platelet shape change. This initial platelet response was blocked by preincubation with DGPP 8:0 or NPSerPA [Rother et al., 2003]. Furthermore, the lipid-rich core or LPA synergized with other platelet stimuli in inducing platelet aggregation. This response required the presence of fibrinogen in the medium. Platelet aggregation in response to core lipids was blocked when platelets were preincubated with LPA antagonists DGGPP 8:0 or NPSerPA.

These results show that the lipid-rich core of human atherosclerotic plaques can induce shape change and trigger aggregation of washed, isolated platelets. This response is mediated by activation of platelet LPA receptors because it could be abolished by LPA receptor antagonists; therefore, it can entirely be attributed to LPA-like molecules contained in the lipid-rich core region [Rother et al., 2003]. Thus, other platelet stimuli such as cholesterol sulfate and oxidized PC molecules with PAF-like activity do not appear to contribute significantly to the platelet-activating effect of the lipid-rich core. Perhaps it is not just a co-incidence that the molecular species of alkyl-GP found in the lipid rich core of human atherosclerotic plaques match the structure–activity relationship of PPAR$\gamma$ activation [Rother et al., 2003; Zhang et al., 2004].

### LPA Stimulates Platelets in Whole Blood

Low nanomolar concentrations of LPA induce shape change of washed platelets, whereas 1,000-fold higher concentrations of LPA are required for the induction of the same response in platelet-rich plasma. This is likely due to the LPA-binding capacity of albumin, which inhibits the platelet-activating effect of LPA [Tigyi and Miledi, 1992; Haserück et al., 2000].

Even higher concentrations of LPA (>20 $\mu$M) are required to induce aggregation of platelet-rich plasma [Schumacher et al., 1979; Tokumura et al., 1981; Haserück et al., 2000]. Until recently it was unknown, whether and through which mechanism could LPA induce platelet shape change and aggregation in whole blood. We found, that LPA at concentrations slightly above plasma levels induces platelet shape change, and aggregation in blood [Haserück et al., 2003]. Alkyl-LPA (16:0) was almost 20-fold more potent than acyl-LPA (16:0). LPA directly induced platelet shape change in blood and platelet-rich plasma obtained from all blood donors. In contrast, LPA-stimulated platelet

aggregation in whole blood was donor-dependent and could be completely blocked by apyrase or antagonists of the platelet ADP-receptors $P2Y_1$ and $P2Y_{12}$. These results indicate a central role for ADP-mediated $P2Y_1$ and $P2Y_{12}$ receptor activation in supporting LPA-induced platelet aggregation. LPA also stimulated the formation of platelet–monocyte aggregates, which are considered as an early marker of acute myocardial infarction. Platelet aggregation and platelet–monocyte aggregate formation stimulated by LPA was insensitive to inhibition by aspirin. The precise identification of LPA receptors mediating aggregate formation of platelets and platelet/monocytes awaits further investigation.

Based on these studies we propose that upon plaque rupture in vivo, LPA molecules present in the lipid-rich core region of the plaque that encounter platelets in circulating blood might be able to induce platelet shape change and platelet aggregation, and hence contribute to the formation of an intra-arterial thrombus with its dire consequences.

### Responses to mox-LDL and LPA in the Intact Non-Injured Vascular Bed—The Role of PPARγ in LPA-Induced Neointima Formation

Recently, LPA has been shown to activate an entirely new level of signaling through the direct activation of the nuclear transcription factor PPARγ. Using transfected RAW264.7 monocytic cells, McIntyre et al. [2003] discovered that LPA was a direct agonist of PPARγ capable of regulating genes that contain PPAR Response Elements (PPRE). Using the CD36 scavenger receptor and the acetyl CoA-oxidase promoters, both of which contain PPREs, they conclusively demonstrated that LPA and fluorinated analogs of LPA, which are 400-times less potent agonists of the EDG-family LPA receptors compared to LPA, upregulated the expression of these genes. They also showed in yeast cells that lack G protein-coupled receptors for LPA, when reconstituted, LPA could activate PPARγ-mediated gene transcription. The LPA-induced transcriptional upregulation of CD36 in macrophages is of very high potential significance because this receptor plays an essential role in lipid accumulation by importing cholesterol and mox-LDL into macrophages leading to foam cell formation.

Concerning the formation of neo-intima, Hayashi et al. [2001] proposed that unsaturated species of LPA present in serum are responsible for VSMC dedifferentiation in vitro mediated by the coordinated activation of the ERK and p38 MAPK pathways. These authors detected $LPA_1$ and $LPA_3$ receptor transcripts in their preparation and noted no changes in the expression pattern during dedifferentiation. Surprisingly, in LPA 18:1 treated cultures caldesmon and calponin mRNA began to decrease as early as 12 h in culture. PTX, or a combination of the MEK inhibitor PD98059, together with either p38 MAPK inhibitor SB203580 or SB2200025 prevented dedifferentiation. PDGF-BB, EGF, and serum also elicited dedifferentiation. No effect was elicited by 16:0 or 18:0 LPA. Hayashi and colleagues reasoned that the unsaturated LPA species present in serum would be solely responsible for the dedifferentiation of cultured VSMC. These authors using gas chromatographic technique detected a total of 27.68 μmol/L LPA in human serum, of which 6.37 μmol/L constituted unsaturated species. This concentration is several fold higher than; moreover their acyl chain composition values did not reveal a dominance of the unsaturated 20:4 and 18:2 acyl species that were found by several other investigators [Saulnier-Blache et al., 2000; Baker et al., 2001; Aoki et al., 2002; Sano et al., 2002]. The lack of dedifferentiation causing effect by LPA 12:0, 14:0, 16:0, 18:0 noted by Hayashi and coworkers is at odds with the expression of $LPA_1$ and $LPA_3$ transcripts, as the $LPA_1$ receptor has been reported to respond to LPA 16:0 and 18:0 [Bandoh et al., 2000]. We have shown that not only unsaturated LPA species but the PPARγ agonist Rosiglitazone also elicits the phenotypic dedifferentiation of VSMC and this response is completely abolished by the irreversible PPARγ antagonist GW9662 [Zhang et al., 2004]. Hayashi et al. [2001] proposed that a sustained activation of the PI3K-Akt signaling axis is a prerequisite for the in vitro maintenance of the differentiated VSMC phenotype. However, PPARγ activates the phosphatase PTEN, which inhibits PI3K signaling [Goetze et al., 2001, 2002a,b] providing an alternative explanation for the phenotypic dedifferentiation that brings PPARγ to center stage as a regulator/modulator of G protein-coupled receptor signaling. These unresolved issues certainly point to the pressing need for further research in this important area of LPA biology.

The mounting evidence collected from in vitro studies prompted Yoshida et al. [2003] to test

LPA for its activities on the non-injured arterial wall. These authors developed a new model in which LPA is infused through the external carotid artery of a rat into a ligated section of the common/internal carotid artery that has been carefully rinsed to remove blood. Low micromolar concentrations of LPA were applied into the vessel for a period of 1 h. It is important to note that the cannula never enters the common carotid, thus there is no mechanical injury to the vessel wall beyond that of the surgical exposure on the adventitial side. As early as 7 days after LPA exposure, there were signs of neointima formation in the common carotid but only to unsaturated acyl species of LPA. These authors found that LPA 18:0 only weakly activated p44/45 ERK and p38 MAPK and did not induce nuclear localization of NF-κB, whereas LPA 18:1 was a strong activator of the two MAP kinases and of NF-κB nuclear translocation. They also noted that a combined pharmacological inhibition of ERK and p38MAPK signaling blocked neointima formation. We investigated the effect of native and mox-LDL using the same model and found that only mox-LDL elicited progressive neointima growth [Zhang et al., 2003, 2004]. We determined that oxidative damage to LDL led to 6-fold increase in the concentrations of alkyl-LPA but acyl-LPA remained the same, although the polyunsaturated species became oxidized. Because of the apparent mismatch between the structure activity relationship of the EDG family LPA receptors and that of the neointima inducing effect limited to unsaturated LPA species, we have investigated the possibility that the neointimal response would be mediated by PPARγ. Our results showed that only unsaturated LPA species and alkyl-LPA but not saturated acyl species or cPA 18:1 were able to activate PPARγ in vivo and in vitro. We obtained several lines of evidence that implicate PPARγ in LPA-induced vascular remodeling:

1) The synthetic and natural PPARγ ligands elicited neointima formation.
2) The irreversible PPARγ antagonist GW9662 completely abolished the neointima response to LPA and other agonists including Rosiglitazone, azeleoyl-phosphatidylcholine and mox-LDL.
3) Fluorinated LPA analogs, which are 400-times less potent agonists of LPA receptors than LPA 18:1 were nearly as effective as LPA 18:1 eliciting neointima; whereas EGF, VEGF, and PDGF were inactive.
4) Whereas LPA GPCR do not show stereoselectivity, PPARγ showed a preference for 1-*O*-octadecenyl-LPA over the un-natural stereoisomer 3-*O*-octadecenyl-LPA. The neointimal response showed the same stereoselectivity as PPARγ.
5) Albumin interferes with the transbilayer movement of LPA in a dose-dependent manner [Tokumura et al., 1992] and blocks the neointimal response. It is for this reason that plasma inhibits the neointima-inducing effect of LPA and Rosiglitazone, whereas serum, that is rich in LPA and is capable of activating LPA GPCR but not PPARγ, remains inactive in eliciting neointimal formation.

Based on these results, we confirm the observations of Yoshida et al. [2003] and propose that activation of PPARγ is sufficient and necessary for the neointimal response [Zhang et al., 2003, 2004]. Taken together, LPA appears to play a dual role in vascular remodeling: one as ligand of GPCR and another as a ligand of PPARγ. The interplay between these two roles will most likely be a topic of intense investigation in the near future.

## Perspective

The cardiovascular system has emerged as a major target of LPA. Based on the evidence available at the present time, we envision that LPA has the definitive potential to be identified as an important mediator of thrombosis and atherogenesis; consequently, experimental therapies targeting LPA receptors and signaling might offer benefits in the treatment of atherosclerosis and cardiovascular diseases. Here we present our vision as a working hypothesis for the role of LPA in atherothrombosis and neointima formation. We posit that LPA is generated during oxidative modification of LDL in the intima, and/or that LPA is released by activated platelets that have been shown to adhere to early atherosclerotic lesions in vivo [Massberg et al., 2002]. LPA will then via LPA receptors activate endothelial cells at the luminal and abluminal side, increase the expression of adhesion molecules for monocytes and enhance endothelial permeability for LDL. In the intima, mox LDL-associated LPA could be taken up by macrophages and VSMCs by a

phospholipid transporter as described in epithelial cells by Boujaoude et al. [2001]. We propose that LPA packaged in LDL is a pathophysiologically relevant ligand that can lead to PPARγ activation. The intracellularly captured LPA and other mox-LDL-associated oxidized lipids activate PPARγ and upregulate the expression of a host of genes with PPRE in their promoters. The increased expression of CD36 in infiltrating macrophages along with the PPARγ-regulated dedifferentiation of VSMC, could be the major driving force behind neointima formation and consequential lipid uptake promoting the development of an atheromatous plaque and the atherosclerotic disease.

The mechanism outlined above includes a feed-forward mechanism: LPA-induces PPARγ-mediated expression of CD36, setting up a continued import of PPARγ ligands into the cell.

The lipid rich core of atherosclerotic plaques is instable and if further destabilized by inflammation could rupture bringing the LPA-rich content in contact with platelets. The lipid-rich core could trigger platelet aggregation, thrombosis and ischemic heart attacks and stroke. Although, there are many reports in the literature that confirm one or another aspect of this hypothesis, many more studies are needed to assess whether these events are interlinked by LPA. Undoubtedly, many new pieces of this interesting puzzle will have to become available from future investigations for a more complete understanding of the cardiovascular effects of LPA.

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