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Review

The role of lipoprotein-associated phospholipase A_2 in atherosclerosis may depend on its lipoprotein carrier in plasma

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ABSTRACT

Platelet-activating factor (PAF) acetylhydrolase exhibits a Ca^{2+}-independent phospholipase A_2 activity and degrades PAF as well as oxidized phospholipids (oxPL). Such phospholipids are accumulated in the artery wall and may play key roles in vascular inflammation and atherosclerosis. PAF-acetylhydrolase in plasma is complexed to lipoproteins; thus it is also referred to as lipoprotein-associated phospholipase A_2 (Lp-PLA_2). Lp-PLA_2 is primarily associated with low-density lipoprotein (LDL), whereas a small proportion of circulating enzyme activity is also associated with high-density lipoprotein (HDL). The majority of the LDL-associated Lp-PLA_2 (LDL-Lp-PLA_2) activity is bound to atherogenic small-dense LDL particles and it is a potential marker of these particles in plasma. The distribution of Lp-PLA_2 between LDL and HDL is altered in various types of dyslipidemias. It can be also influenced by the presence of lipoprotein (a) [Lp(a)] when plasma levels of this lipoprotein exceed 30 mg/dl. Several lines of evidence suggest that the role of plasma Lp-PLA_2 in atherosclerosis may depend on the type of lipoprotein particle with which this enzyme is associated. In this regard, data from large Caucasian population studies have shown an independent association between the plasma Lp-PLA_2 levels (which are mainly influenced by the levels of LDL-Lp-PLA_2) and the risk of future cardiovascular events. On the contrary, several lines of evidence suggest that HDL-associated Lp-PLA_2 may substantially contribute to the HDL antiatherogenic activities. Recent studies have provided evidence that oxPL are preferentially sequestered on Lp(a) thus subjected to degradation by the Lp(a)-associated Lp-PLA_2. These data suggest that Lp(a) may be a potential scavenger of oxPL and provide new insights into the functional role of Lp(a) and the Lp(a)-associated Lp-PLA_2 in normal physiology as well as in inflammation and atherosclerosis. The present review is focused on recent advances concerning the Lp-PLA_2 structural characteristics, the molecular basis of the enzyme association with distinct lipoprotein subspecies, as well as the potential role of Lp-PLA_2 associated with different lipoprotein classes in atherosclerosis and cardiovascular disease.

1. Introduction

PAF (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a biologically active phospholipid that expresses several proinflammatory activities and is involved in various pathophysiological conditions including atherogenesis [1,2]. PAF is hydrolyzed and inactivated by PAF-acetylhydrolase, a Ca^{2+}-independent, phospholipase A_2 (PLA_2) [3]. Two major forms of PAF-acetylhydrolase have been described, the secreted (plasma) and the intracellular (cytosolic) forms [4,5]. The plasma PAF-acetylhydrolase is also referred to as GVIIA PLA_2, owing to its hierarchical position in the PLA_2 VIIA superfamily [6]. PAF-acetylhydrolase circulates in plasma in active form bound on lipoproteins [7–9] and due to this property it is named as lipoprotein-associated phospholipase A_2 (Lp-PLA_2) [9]. In human plasma Lp-PLA_2 is mainly associated with low-density lipoprotein (LDL) as well as with high-density lipoprotein (HDL). Lp-PLA_2 is also bound to very low-density lipoprotein (VLDL) and to intermediate density lipoprotein (IDL) as well as to lipoprotein (a) [Lp(a)] [3]. More recently, we provided strong evidences that except for lipoproteins, another carrier of Lp-PLA_2 in human plasma is platelet-borne microparticles (PMPs) [10]. It is well documented by in vitro studies, by studies in animal models in vivo as well as by clinical trials that Lp-PLA_2 is implicated in atherosclerosis and cardiovascular disease. However the precise pathophysiological role of this enzyme in plasma as well as in the artery wall needs further clarification. In this regard, several studies have suggested that this role may be differentiated according to the type of the lipoprotein carrier with which Lp-PLA_2 circulates in plasma. This review discusses current advances on the structural and catalytic features of plasma Lp-PLA_2, the enzyme association with distinct lipoprotein subspecies, as well as on the potential role of Lp-PLA_2 in atherosclerosis and cardiovascular disease.

2. Structural and catalytic features of plasma Lp-PLA_2

The human plasma Lp-PLA_2 is produced as a protein composed of 441 amino acid residues and is secreted into the circulation after cleavage of a hydrophobic signal peptide at the cleavage position, Ala-

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17/Val-18 [11,12]. Lp-PLA_2 is N-glycosylated at two asparagine residues, Asp-423 and Asp-433 and contains about 9 kDa of a heterogeneous sugar chain(s), involving sialic acid [9,13]. The primary structure of Lp-PLA_2 is unique with the exception of the Gly-His-Ser-Phe-Gly consensus sequence in the enzyme catalytic site, which is similar to that found in serine esterases and lipases. Ser-273 of the Gly-His-Ser-Phe-Gly motif as well as Asp-296 and His-351 constitute the enzyme catalytic triad [12]. Thus Lp-PLA_2 is markedly inhibited by serine esterase inhibitors such as diisopropylfluorophosphate and Pefabloc [14,15]. The linear orientation and spacing of Ser-273, Asp-296 and His-351 are consistent with the α/β hydrolase conformation [16]. The above observations were confirmed by the enzyme crystal structure, which was very recently solved from X-ray diffraction data collected to a resolution of 1.5 Å [17]. According to this new data, Ser-273 is located at the N terminus of an α-helix and on the Gly-His-Ser-Phe-Gly motif. The amide nitrogens of Phe-274 and Leu-153 are well poised to stabilize the negative charge of a tetrahedral intermediate of the reaction mechanism, thereby acting as the oxyanion hole of the enzyme. The other two catalytic triad residues are appropriately positioned to activate the nucleophilic Ser-273 for catalysis; Asp-296 is located on the C-terminal end of a β-sheet, and His-351 is located at the N terminus of an α-helix. Like other lipases, the catalytic triad lies within a hydrophobic pocket that is oriented toward its substrate. Notably, the orientation of the catalytic triad and hydrophobic residues Leu-153 and Phe-274 were previously predicted based on modeling from the distantly related *S. exfoliatus* lipase structure [16]. From previous functional and kinetic characterization of Lp-PLA_2, it was suggested that the active site would allow substrates to enter from lipoproteins as well as from the aqueous phase [18,19]. These findings are consistent with the placement of the enzyme active site, which sits just above the interface-binding surface (that accesses the lipoprotein particle) and faces the aqueous phase [17]. There are two α-helices that help the enzyme associate with lipoproteins. One α-helix (114–126) that had been predicted to be a component of the interfacial binding surface of Lp-PLA_2 that accesses the LDL particles (this is described below). In addition to the interface positioning of several hydrophobic residues, this α-helix has also His-114, an apparently polar and charged residue that is predicted to be buried in the hydrophobic lipid phase, as well as a Met-117 residue that has been shown to play an oxidative inactivation role [20]. The second α-helix (residues 362–369) has hydrophobic residues positioned to insert into the hydrophobic portion of the aqueous–lipid interface [17]. This region has been recently shown to influence Lp-PLA_2 binding to HDL (this is described below). Finally, an acidic patch of 10 carboxylate residues (Asp-374, Asp-376, Asp-382, Asp-401, Asp-403, Asp-406, Glu-410, Asp-412, Asp-413, and Glu-414) and a neighboring basic patch of three residues (Lys-55, Arg-58, and Lys-363) are suggested to play a role in HDL/LDL enzyme partitioning [17].

Lp-PLA_2 is specific for short acyl chains (up to 9 methylene groups) at the *sn-2* position of the phospholipid substrate. The first substrate described for Lp-PLA_2 was PAF, which is hydrolyzed into lyso-PAF and acetate (Fig. 1). An important breakthrough in Lp-PLA_2 research was the finding that this enzyme can also effectively hydrolyze oxidized phospholipids (oxPL) produced by non-enzymatic radical-mediated oxidation of phosphatidylcholines (PC) containing an *sn-2* polyunsaturated fatty acyl residue [21,22]. oxPL are substrates for Lp-PLA_2, despite the fact that they contain longer than 9 carbon atom chains. This is due to the fact that the ω-end of their *sn-2* peroxidized fatty acyl residues contains aldehydic or carboxylic moieties [22]. Some of oxPL undergo chain fragmentation in their peroxidized acyl chains and become truncated. These oxPL are hydrolyzed more efficiently by Lp-PLA_2 than oxPL containing nonfragmented-peroxidized chains [23]. Hydrolysis of oxPL results in the generation of oxidized free fatty acid (oxFFA) and lyso-PC (Fig. 1). A wide spectrum of oxPL has been

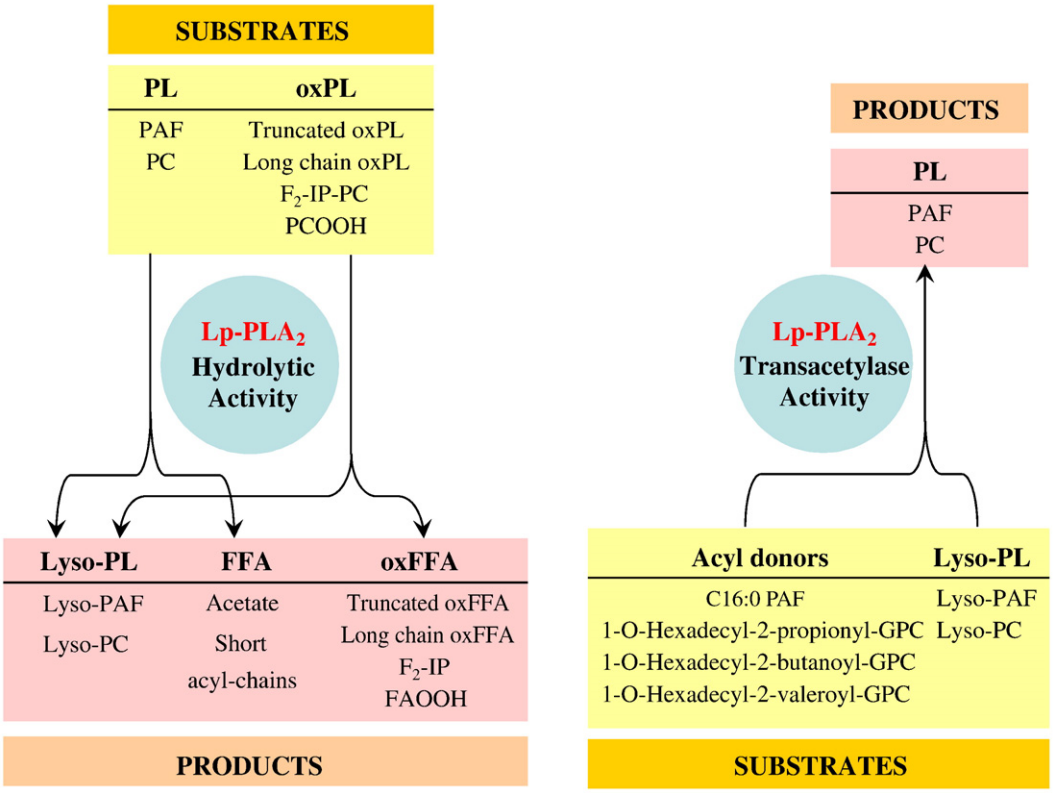

Fig. 1. Hydrolytic and transesterification reactions cartalyzed by Lp-PLA_2. F_2-IP = F_2-isoprostane(s), FFA = free fatty acid(s), F_2-IP-PC(s) = phoshatidylcholine F_2-isoprostane(s), FAOOH(s) = fatty acid hydroperoxide(s), Lyso-PC = lyso-phosphatidylcholine, oxFFA = oxidized free fatty acid(s), oxPL = oxidized phospholipid(s), PAF = Platelet-activating factor, PC = phosphatidylcholine, PCOOH = 1-palmitoyl-2-hydroperoxyoleoyl-sn-glycero-3-phosphocholine, PL = phospholipid(s), and -GPC = glycerophosphorylcholine.

identified in oxidized LDL (oxLDL) particles; many of them express biological activities and play key roles in several aspects of atherogenesis [24]. Lp-PLA_2 also exhibits hydrolytic activity towards long fatty acyl chain phospholipid hydroperoxides such as 1-palmitoyl-2-hydroperoxyoleoyl-sn-glycero-3-phosphocholine (PCOOH) [25] (Fig. 1). Furthermore, it was recently demonstrated that Lp-PLA_2 could catalyze the hydrolysis of phospholipids containing F_2-isoprostanes esterified at the *sn-2* position (F_2-IP-PC) in vitro and in vivo [26] (Fig. 1). Importantly, Lp-PLA_2 is the only PLA_2 in plasma that can catalyze this reaction since subjects deficient in Lp-PLA_2, owing to a mutation near the active site, lacked the ability to release free isoprostanes (F_2-IP) from esterified precursors [26]. Substrates for Lp-PLA_2 are also other lipid esters that are partially soluble in the aqueous phase [18], including short-chain diacylglycerols, triacylglycerols, and acetylated alkanols, and also it displays a PLA_1, activity [19]. In addition to the lipase and esterase activities of Lp-PLA_2, this enzyme also exhibits a transacetylase activity [27]. This activity is present on LDL and transfers acetate as well as short-chain fatty acids from PAF and its ether/ester-linked analogues to ether/ester-linked lysophospholipids [28] (Fig. 1). The Lp-PLA_2-catalyzed transacetylase activity has not been adequately studied, however this activity was recently detected in the artery wall, suggesting that it may play a role in regulating the levels of PAF and related analogues as well as lysophospholipids during atherogenesis (discussed below) [29]. Finally, it was recently demonstrated that Lp-PLA_2 is responsible for the desoctanoylation of Ghrelin associated with LDL; a peptide hormone produced predominantly by the stomach and stimulates food intake and growth hormone secretion [30]. Although most of the Lp-PLA_2 substrates described above are generated during oxidative stress, the enzyme itself is susceptible to oxidative inactivation [31–33]. Thus, the progressive Lp-PLA_2 inactivation during oxidation may facilitate the accumulation of enzyme substrates formed under oxidative stress [31]. The enzyme inactivation is irreversible and it was recently shown that one of the primary targets of oxidation is Met-117 [20]. Furthermore, peroxynitrite ($ONOO^-$) is one of the species that inactivates Lp-PLA_2 inducing tyrosine nitration, possibly at residues Tyr-307 and Tyr-335 [20].

3. Secretion of Lp-PLA_2 and association of with plasma lipoproteins

The human plasma Lp-PLA_2 is produced and secreted from hematopoietic stem cell-derived cells (monocyte/macrophages, mast cells, T-lymphocytes, platelets) [10,34–37]. Secretion of Lp-PLA_2 is affected by the state of cellular differentiation and is also regulated by several exogenous stimuli such as PAF, LPS, a variety of cytokines and steroid hormones. Importantly, the ability of pro- and anti-inflammatory agents to modulate Lp-PLA_2 expression seems to be dependent on the state of cellular differentiation [38]. Transcriptional control mechanisms contribute to the ability of exogenous stimuli to modulate Lp-PLA_2 expression. The Lp-PLA_2 promoter contains a set of GC-rich motifs surrounding the transcription start site, a feature characteristic of promoters that regulate the expression of housekeeping genes [39,40]. A number of elements in the human Lp-PLA_2 promoter region have been identified and MS2 as well other differentiation-induced transcription factors are likely involved in the regulation of expression of the *Lp-PLA_2* gene [39]. Furthermore, the binding of the transcription factors Sp1 and Sp3 to both canonical and non-canonical Sp-binding sites localized within the minimal promoter region participates in the constitutive expression of human plasma *Lp-PLA_2* gene in macrophages [40]. Interestingly, the levels of Sp1 are high in developing hematopoietic cells and may explain the elevated expression of Lp-PLA_2 associated with differentiation of monocytes into macrophages [40]. In addition to its contribution to basal expression, Sp1 is required for the ability of LPS to transcriptionally activate the *Lp-PLA_2* gene [41].

Circulating Lp-PLA_2 derives primarily from cells of the hematopoietic lineage, ie cells that do not produce lipoproteins. This was shown by Asano et al. [34] who reported that when a Japanese patient with normal plasma Lp-PLA_2 activity received an allogenic bone marrow transplant from a donor who was homozygous for the Lp-PLA_2 mutation V279F (which results in complete Lp-PLA_2 inactivation), transplantation resulted in lack of Lp-PLA_2 activity in the recipient's serum. However, this phenomenon was not observed in patients who received bone marrow from donors with normal serum Lp-PLA_2 levels or from heterozygotes for the above Lp-PLA_2 mutation, as the recipients displayed normal or half normal serum Lp-PLA_2 activity, respectively [34]. Thus, release of Lp-PLA_2 occurs independently of lipoprotein secretion; the enzyme subsequently associates with these particles [42]. Consistent with this hypothesis, Lp-PLA_2 activity in plasma of individuals with HDL deficiency (Tangier disease) is higher than that of normal subjects [43], whereas individuals with abetalipoproteinemia have normal or slightly subnormal Lp-PLA_2 activity [44,45]. More recently, we provided evidence that another carrier of Lp-PLA_2 in human plasma could be platelet-borne microparticles (PMPs) [10].

3.1. Association of Lp-PLA_2 with LDL

In normolipidemic human plasma with no detectable Lp(a) levels, Lp-PLA_2 activity is mainly associated with LDL (LDL-Lp-PLA_2) [3]. A major role in the association of Lp-PLA_2 with LDL is played by the apolipoprotein B-100 (apoB-100) moiety. Especially, the carboxyl terminus of this apolipoprotein (residues 4119–4279) is required for the interaction with Lp-PLA_2. On the other hand, residues Tyr-205, Trp-115 and Leu-116 of Lp-PLA_2 and to lesser extent Met-117, are critical for enzyme association with LDL [46]. Among the LDL subspecies, the enzyme activity preferentially associates with small-dense LDL (sdLDL) [47,48]. Other studies have reported that most of LDL-Lp-PLA_2 activity is associated with electronegative subfraction of LDL [LDL(−)] which represents the 8% and 15.6% of plasma LDL in normolipidemic and familial hypercholesterolemic subjects, respectively [49]. This observation coincides with the preferential association of the enzyme with sdLDL, because LDL(−) is abundant in sdLDL [50]. More recently we demonstrated [51] that one molecule of Lp-PLA_2 corresponds to 100 particles of sdLDL and to 4000 particles of large buoyant LDL subfractions. It should be noted however that the Lp-PLA_2 activity in sdLDL is much less than that expected from the enzyme mass carried by these particles. Indeed, the specific activity of the Lp-PLA_2 associated with LDL-5 is 2- to 4-folds lower than that associated with the other apoB-containing lipoprotein subfractions [51].

Total plasma Lp-PLA_2 activity is positively correlated with sdLDL-cholesterol mass and proportion and negatively with mean LDL particle size. Similar correlations exist between sdLDL parameters and the activity or mass of the plasma non-HDL-associated Lp-PLA_2, calculated by subtracting the HDL-associated Lp-PLA_2 (HDL-Lp-PLA_2) activity or mass from the total plasma enzyme activity or mass, respectively [51]. Importantly, the plasma Lp-PLA_2 activity and the non-HDL-associated Lp-PLA_2 activity are the second best predictors of the presence of sdLDL particles in human plasma after serum triglyceride concentrations. Furthermore, at serum triglyceride concentrations >1.356 mM, the total plasma Lp-PLA_2 activity as well as the non-HDL-Lp-PLA_2 activity significantly add to the prediction of the presence of sdLDL in plasma [51].

3.2. Association of Lp-PLA_2 with HDL

A small proportion of Lp-PLA_2 activity in normolipidemic human plasma with no detectable Lp(a) levels, circulates bound on HDL [3]. It was initially suggested that Lp-PLA_2 protein is lacking in HDL and HDL-Lp-PLA_2 activity may derives from paraoxonase-1 (PON1) [52],

an enzyme primarily associated with HDL [53]. In this regard it was demonstrated that among the various hydrolytic activities expressed by PON1 this enzyme is also capable to hydrolyze specific oxPL [54] such as truncated *sn-2* fatty acyl groups of phospholipids from oxidized HDL [55]. Furthermore, PON1 exhibits PAF hydrolytic activity [52]. In contrast to these results, it was subsequently demonstrated that the above mentioned Lp-PLA_2-like activities expressed by PON1 [52,54,55] do not arise from PON1 itself but rather from a trace contamination of Lp-PLA_2 existing in the isolated and purified PON1 protein used in the above enzymatic assays [56]. These later studies further showed that Lp-PLA_2 is the sole phospholipase A_2 of HDL and that PON1 has no phospholipase activity toward PAF or oxidized phospholipids [56]. In accordance to this observation, it was demonstrated that recombinant PON1 does not exhibit significant peroxidatic or hydrolytic activity towards PCOOH [25], whereas recombinant Lp-PLA_2 readily hydrolyzes PCOOH, releasing stoichiometric amounts of oleic acid hydroperoxide [25]. Furthermore, recombinant Lp-PLA_2, but not PON1, catalyzes PLOOH hydrolysis in peroxidized LDL [25]. These findings suggest that the phospholipid hydroxyperoxide hydrolyzing/detoxifying role often attributed to PON1 on the basis of in vitro studies with enzyme isolated from HDL [57], actually reflect the activity of Lp-PLA_2, which exists as a contamination in the preparations of purified PON1 [56].

The domains required for association of Lp-PLA_2 with HDL have been recently identified [58]. Sequence comparisons among species combined with domain-swapping and site-directed mutagenesis studies have led to the identification of a string of C-terminal residues His-367, Met-368, Leu-369, and Lys-370 (HMLK) unique to human Lp-PLA_2 that is necessary for binding to HDL. Interestingly, this region is not conserved among Lp-PLA_2 of various species, suggesting that Lp-PLA_2 interacts with HDL particles in a manner that is unique to each species [58]. A prominent role seems to play Met-368 and Leu-369 residues whereas a more moderate contribution may have His-367 and Lys-370 residues. It should be noted that Met-368 and Leu-369 are necessary but not sufficient for binding to HDL, as the guinea pig enzyme, which harbors both of these residues, but not the complete string, do not bind to the lipoprotein. These results suggest that His-367 and Lys-370 either directly participate in Lp-PLA_2 association with HDL or contribute to the formation of a binding pocket that optimizes interaction of Met-368 and Leu-369 with the lipoprotein [58]. However, it is possible that additional regions of human Lp-PLA_2 are required for interaction with HDL. In this regard, we have previously shown that removal of the carbohydrate content of the macrophage-derived Lp-PLA_2 (which represents one of the major sources of the enzyme pool in plasma) enhances enzyme association with both HDL-2 and HDL-3 subfractions. These results provide evidence that a factor contributing to the preferential association of Lp-PLA_2 with LDL versus HDL in human plasma is the enzyme glycosylation [59].

Among the apoA-I-containing lipoproteins Lp-PLA_2 is preferentially associated with the very high-density lipoprotein-1 subfraction (VHDL-1) [48], alternatively denoted as HDL3c [60]. One molecule of Lp-PLA_2 corresponds to 10 particles of HDL-3c or to 1500 particles of the HDL-3a subfraction. Importantly, the specific activity of Lp-PLA_2 associated with the HDL-3c subfraction is 2- to 6-folds lower than in the other HDL subfractions [51]. Furthermore, in plasma with normal LDL-cholesterol levels, the Lp-PLA_2 activity associated with total HDL (HDL-Lp-PLA_2) represents the $4.9 \pm 0.8\%$ of the total plasma enzyme activity, whereas the HDL-Lp-PLA_2 mass represents the $28.5 \pm 2.0\%$ of total plasma enzyme mass. Thus, the HDL-Lp-PLA_2 activity does not significantly contribute to the total plasma enzyme activity, whereas the HDL-Lp-PLA_2 mass significantly influences the total plasma enzyme mass [51]. It should be emphasized that according to our previously published results, the method used for the determination of Lp-PLA_2 mass may not detect all active enzymes in LDL, a phenomenon not observed for HDL. This suggests that structural differences among lipoprotein species may significantly influence the determination of enzyme mass, a hypothesis that needs further investigation [61].

3.3. Association of Lp-PLA_2 with Lp(a)

The distribution of Lp-PLA_2 between LDL and HDL can be influenced by the presence of Lp(a) when plasma levels of this lipoprotein exceed 30 mg/dl [62]. Interestingly, it was demonstrated that Lp(a) is enriched in Lp-PLA_2 since it contains 1.5 to 2-folds higher enzyme mass [63] and several-folds greater Lp-PLA_2 activity compared with LDL when assayed at equimolar protein concentrations [64]. The Lp(a)-associated Lp-PLA_2 [Lp(a)-Lp-PLA_2] protein is highly glycosylated [63] and it is susceptible to oxidation in vitro [62]. The major role in the attachment of Lp-PLA_2 on the Lp(a) particles, is played by the apoB-100 moiety of Lp(a), whereas the enzyme does not bind to apolipoprotein (a) [62,64]. Importantly, there are marked differences in the enzyme catalytic properties among the various Lp (a) isoforms, the small isoforms exhibiting higher apparent Km and Vmax values, compared to large ones, suggesting that the apo(a) may influence the association of Lp-PLA_2 with Lp(a), although it does not bind the enzyme itself [62].

3.4. Lp-PLA_2 association with platelet-derived microparticles (PMPs)

One of the cells that contribute to the plasma pool of Lp-PLA_2 are platelets [37,65]. Indeed, we have demonstrated that human platelets contain 2 types of PAF-acetylhydrolase, the intracellular type II, which is localized in the cytosol and represents the 75% of total enzyme activity as well as the plasma type (Lp-PLA_2) which is not glycosylated, is associated with the cell membranes and represents the 25% of total platelet enzyme activity [10]. Furthermore, we showed that platelets contain high levels of the Lp-PLA_2 mRNA, suggesting that these cells may synthesize de novo Lp-PLA_2 however, translation of the mRNA may be independent of the platelet activation state. A substantial proportion of the platelet Lp-PLA_2 is secreted during thrombin-induced aggregation, in parallel to the shedding of PMPs observed during platelet aggregation. However platelet aggregation is not required either for Lp-PLA_2 secretion or for PMPs production. Our results suggest that the secretion of Lp-PLA_2 from platelets depends on the shedding of PMPs from their surface, and this could be the major mechanism for Lp-PLA_2 secretion on platelet activation. This is supported by the observation that the Ca^{2+}-ionophore A23187, which induces platelet vesiculation but not platelet aggregation [66], promoted Lp-PLA_2 secretion in parallel to the production of PMPs. Furthermore, calpeptin, a specific inhibitor of calpain and thus PMPs formation, significantly inhibited the generation of PMPs and the secretion of Lp-PLA_2. Thus, vesiculation of platelet membrane and formation of PMPs are necessary for Lp-PLA_2 secretion from platelets. Importantly, by using an immunocaptured ELISA method, we demonstrated that not only PMPs generated on platelet activation in vitro but also PMPs existing in plasma (CD61 positive particles) of normolipidemic volunteers contain Lp-PLA_2 [10]. It should be noted that neither circulating endothelial-derived microparticles nor monocyte-borne microparticles contain Lp-PLA_2 activity.

3.5. Dyslipidemias affect the Lp-PLA_2 levels and partition among plasma lipoproteins

Abnormalities in lipoprotein metabolism significantly affect the Lp-PLA_2 levels in plasma and alter the distribution of Lp-PLA_2 among plasma lipoproteins. In primary hypercholesterolemia (Type IIA dyslipidemia), the LDL-Lp-PLA_2 activity is increased in parallel with the increase in the severity of hypercholesterolemia and the highest levels of enzyme activity are observed in homozygous familial hypercholesterolemia whereas the lowest are seen in patients with non-familial hypercholesterolemia. By contrast, the HDL-Lp-PLA_2

activity is not significantly influenced [67]. These results suggest that in subjects with normal or increased LDL-cholesterol levels in plasma, an important determinant of plasma Lp-PLA_2 levels as well as the enzyme distribution among plasma lipoproteins is the rate of removal of LDL from the circulation, an observation, which accords with previously published results [68]. Patients with combined hyperlipidemia (Type IIB dyslipidemia) also exhibit increased total plasma and LDL-Lp-PLA_2 activities. In these patients, Lp-PLA_2 activity in the triglyceride-rich VLDL and IDL is also increased whereas the HDL-Lp-PLA_2 and HDL-cholesterol levels are reduced [69]. Furthermore, patients with primary hypertriglyceridemia (Type IV dyslipidemia) exhibit increased plasma Lp-PLA_2 activity, which is mainly due to the elevation in Lp-PLA_2 activity associated with VLDL and IDL. The LDL-Lp-PLA_2 activity is not altered in this type of dyslipidemia, whereas the HDL-Lp-PLA_2 activity and HDL-cholesterol levels are reduced [60]. A common feature of the above dyslipidemias is an altered distribution of enzyme activity among apoB- and apoA-I-containing lipoproteins, which is characterized by a reduction in the ratio of HDL-Lp-PLA_2 to total plasma enzyme levels [60,67–69]. The alterations in the plasma Lp-PLA_2 levels as well as the enzyme distribution among plasma lipoproteins observed in various dyslipidemias is at least partially restored either by low-calorie diet associated with weight loss [70] or after treatment with orlistat [71] or hypolipidemic drugs, ie statins (atorvastatin, simvastatin, rosuvastatin, fluvastatin), fenofibrate, ezetimibe, niacin or their combination. All these hypolipidemic drugs reduce Lp-PLA_2 in plasma by decreasing the LDL-Lp-PLA_2 levels. Furthermore, fenofibrate significantly increases the HDL-Lp-PLA_2 in plasma [61,71–82].

4. Role of Lp-PLA_2 in atherosclerosis and cardiovascular disease

4.1. Antiatherogenic and proatherogenic effects of LDL-Lp-PLA_2

The LDL-Lp-PLA_2 is the major determinant of plasma Lp-PLA_2 levels. A substantial body of peer-reviewed studies in Caucasian population has supported Lp-PLA_2 as a cardiovascular risk marker independent of and additive to traditional risk factors [83]. Increased Lp-PLA_2 levels in plasma approximately double the risk for primary and secondary cardiovascular events. Over 25 prospective epidemiologic studies have demonstrated the association of elevated Lp-PLA_2 levels with future coronary events and stroke, 11 of 12 prospective studies have shown a statistically significant association between elevated Lp-PLA_2 and primary coronary or cardiovascular events, 12 of 13 have shown a statistically significant association with recurrent coronary or cardiovascular events, and 6 studies have shown a positive association with stroke. Thus Lp-PLA_2 (mainly LDL-Lp-PLA_2) should be viewed today as an important cardiovascular risk marker whose utility is as an adjunct to the major risk factors to adjust absolute risk status and thereby modify low-density lipoprotein cholesterol goals. The low biologic fluctuation and high vascular specificity of Lp-PLA_2 makes it possible to use a single measurement in clinical decision-making, and it also permits clinicians to follow the Lp-PLA_2 marker serially [84].

However it remains to be established whether this enzyme is a novel biomarker or a causal mediator for atherosclerotic diseases. Indeed the biological role of LDL-Lp-PLA_2 is controversial since anti- or proatherogenic functions of this enzyme have been proposed. The antiatherogenic properties of Lp-PLA_2 are attributed to the catabolism of PAF and oxPL exhibiting proatherogenic activities [85]. PAF activates various cells participating in atherogenesis, it is synthesized locally at the site of endothelial injury and it accumulates in the atherosclerotic plaques of subjects with advanced coronary artery disease, suggesting that this phospholipid mediator actively participates in the pathophysiology of atherosclerosis [reviewed in Ref 85]. In addition, PAF itself as well as proinflammatory and vasoactive oxidized phospholipids are formed in LDL during oxidation and are believed to play central roles in the formation of atherosclerotic plaques. To this end, a number of studies have shown that minimally modified LDL containing oxPL induce chemotaxis and monocyte adhesion to endothelial cells [86,87]. Interestingly, mildly oxLDL and other apoB-containing lipoproteins depleted of Lp-PLA_2 activity, exhibit increased stimulation of monocyte chemotaxis and adhesion compared with Lp-PLA_2 non-depleted lipoproteins, an activity which is blocked by PAF receptor antagonists [88]. Furthermore, minimally modified LDL from Japanese subjects with a deficiency of plasma Lp-PLA_2 activity is markedly more bioactive compared with LDL from control subjects with normal plasma Lp-PLA_2 [88]. An interesting observation recently published is that a proportion of the oxidized PC formed during LDL oxidation is not detectable; possibly as a result of protein adduct formation [23]. Indeed the formation of lipid–protein adducts with proinflammatory properties that contribute to atherogenesis is known to accompany LDL oxidation [89]. OxLDL is also immunogenic and the oxPL and lyso-PC content plays important role in oxLDL antigenicity participating in the formation of various epitopes, which are recognized by a wide variety of autoantibodies. Such antibodies are present in serum of healthy individuals and in various disease states including coronary artery disease and hypertension [90–93]. In this regard we have demonstrated that extent of LDL oxidation and the LDL-Lp-PLA_2 activity through its capability to hydrolyze oxPL into lyso-PC may play important roles in modulating the epitopes formed on oxLDL and consequently in the oxLDL immunogenicity [94]. Furthermore, we have recently shown that a short-term immune response against oxLDL forms enriched in lyso-PC and a chronic immune response against oxLDL forms enriched in oxPL are observed in patients presented with an acute coronary syndrome without persistent elevation of the ST segment (NSTE-ACS) and with undetectable serum levels of Lp(a). This suggests that the LDL-Lp-PLA_2 may play an important role in modulating the short-term and chronic immune response to oxLDL, observed after an NSTE-ACS [95]. Further studies are required to establish whether the modulation of the oxLDL antigenicity by LDL-Lp-PLA_2 is an antiatherogenic or a proatherogenic effect of this enzyme.

The proinflammatory and proatherogenic role of Lp-PLA_2 is supported by the observation that during the hydrolysis of oxPL, this enzyme generates lyso-PC and oxFFA both of which exhibit several proatherogenic effects [96–98]. Recently it was demonstrated that following the induction of diabetes and hypercholesterolemia in a porcine model, plasma Lp-PLA_2 activity was rapidly increased accompanied by elevated Lp-PLA_2 mRNA expression by peripheral blood mononuclear cells and in coronary arteries. These changes were paralleled by increased inflammatory responses by circulating peripheral blood mononuclear cells (ICAM-1, IL-6), in coronary tissues (ICAM-1, VCAM-1), and the subsequent accumulation of inflammatory cells [99]. Lp-PLA_2 inhibition by the specific inhibitor (SB677116) abrogated the inflammatory response [99]. In support of the proatherogenic action of Lp-PLA_2, a recent in vivo study reported that atherosclerotic plaque in human coronary arteries in vivo is associated with local coronary production of Lp-PLA_2. Also there was net production of Lp-PLA_2 in patients with coronary atherosclerosis versus patients with no atherosclerosis. Local coronary production of lyso-PC was also observed and it was associated with endothelial dysfunction. These results support the role for Lp-PLA_2 in the mechanism of regional vascular inflammation and atherosclerosis in humans [100].

A further support of the proatherogenic role of LDL-Lp-PLA_2 is derived from its preferential association with sdLDL [3], the most atherogenic LDL particles [101]. The enrichment of sdLDL with Lp-PLA_2 has as a consequence the enhanced production of lyso-PC during oxidation, in both normolipidemic and hypercholesterolemic patients [47,102]. In addition, we have previously reported that in the presence of exogenous lyso-PAF, sdLDL-associated Lp-PLA_2 can lead to transformation of inactive lyso-PAF to proinflammatory PAF through the transesterification reaction [28]. Thus the Lp-PLA_2 associated with the

sdLDL particles may lead to their enrichment in the proinflammatory and proatherogenic phospholipids lyso-PC and PAF. Other studies have demonstrated that Lp-PLA_2 may significantly contribute to the atherogenicity of LDL(−) subfraction [103,104]. Finally, the role of LDL-Lp-PLA_2 as a causal mediator for atherosclerotic diseases is supported by recent clinical study aiming to explore the effects of treatment with darapladib a direct Lp-PLA_2 inhibitor that induces a sustained inhibition of plasma Lp-PLA_2 activity [105] on coronary plaque deformability, composition, and size. The results showed that despite adherence to a high level of standard-of-care treatment, the necrotic core continued to expand among patients receiving placebo. In contrast, Lp-PLA_2 inhibition with darapladib prevented necrotic core expansion, a key determinant of plaque vulnerability. These findings suggest that Lp-PLA_2 inhibition may represent a novel therapeutic approach [106].

4.2. Lp-PLA_2 in the artery wall

Almost all of the studies attempting to investigate the role of Lp-PLA_2 in atherosclerotic diseases in humans have been conducted either in vitro in various cell systems or ex vivo by measuring the enzyme mass or/activity in the circulation and then correlating the obtained results with the extent of atherosclerosis and related cardiovascular syndromes. However the conditions in the artery wall and particularly in the atherosclerotic plaques may be significantly different to those in plasma (ie predominance of macrophages over monocytes, increased oxidative stress, mostly modified over native lipoproteins, apoptotic phenomena, chronic inflammatory reactions, etc. Thus, the knowledge concerning the expression levels of Lp-PLA_2 (mass and activity) as well as the levels of the enzyme substrates and products in relation to the above conditions as well as in relation to the plaque morphology and type, could provide invaluable information concerning the pathophysiological role of Lp-PLA_2 in atherosclerosis. Previous work using combined in situ hybridization and immunocytochemistry, detected Lp-PLA_2 (mRNA and protein) in macrophages in both human and rabbit aortic atherosclerotic lesions. Importantly, Lp-PLA_2 expression was greatest in advanced lesions [107]. Subsequently, the localization and distribution of Lp-PLA_2 protein in human coronary atheroma was studied. Results showed that Lp-PLA_2 is expressed in advanced ruptured and rupture-prone lesions designated as thin-cap fibroatheromas [108]. Immunostaining of lesions defined as pathologic intimal thickening or fibroatheromas showed only minimal reactivity to Lp-PLA_2 and when present, it was mostly localized to the lipid pool or necrotic core (abundant in lipids and oxidation products), respectively. In contrast, thin-cap fibroatheromas and ruptured plaques showed extensive Lp-PLA_2 accumulation closely associated within the areas of the necrotic core and surrounding macrophages including those in the fibrous cap. Although Lp-PLA_2 was prominent in macrophages of advanced lesion, there was minimal expression in smooth muscle cells. Double staining experiments showed localization of Lp-PLA_2 within apoptotic macrophages suggesting that its products either represent a marker of apoptosis or they might play a causal role in the induction of cell death. Together, these findings suggest that Lp-PLA_2 may be closely linked with the progression and vulnerability of human coronary atheroma [108]. More recently immunochemical analysis for Lp-PLA_2 in human atherosclerotic aorta and nonatherogenic mammary arteries, showed a diffused and heterogeneous enzyme expression in macrophages of atherosclerotic aortic intima. Lp-PLA_2 was also expressed in media of both atherosclerotic aorta and healthy mammary arteries [29]. The enzyme expression was higher in aortic lesions than in the mammary arteries of the same patient. In the same study, the enzyme hydrolytic and transacetylase activities were significantly higher in the atherosclerotic aortic samples compared to the healthy mammary aortic samples. Importantly, in the healthy mammary arteries the Lp-PLA_2 hydrolytic activity was significantly higher than the transacetylase activity and this was accompanied by low PAF levels, whereas in the atherosclerotic aortic samples the transacetylase activity was slightly higher than the hydrolytic activity accompanied by lyso-PAF accumulation and increased PAF formation [29]. Thus the imbalance between the Lp-PLA_2 hydrolytic and transacetylase activity (in favor to the transacetylase activity) may contribute to the progression of atherosclerosis [29].

4.3. Antiatherogenic effects of the HDL-Lp-PLA_2

The first evidences for the antiatherogenic role of the HDL-Lp-PLA_2, were provided from studies in vitro, which demonstrated that this enzyme significantly contributes to the HDL-mediated inhibition of cell stimulation induced by oxLDL [109]. Subsequently, several in vivo studies supported the anti-inflammatory and antiatherogenic role of the HDL-Lp-PLA_2. However these studies have been performed in rodents and rabbits where the vast majority of plasma Lp-PLA_2 is associated with HDL. The difference between these animals and humans in the distribution of Lp-PLA_2 between LDL and HDL has been attributed to species differences in amino acid sequences in both Lp-PLA_2 and apoB [46]. Adenoviral transfer of the human plasma *Lp-PLA_2* gene in atherosclerosis-prone apoE−/− mice increased Lp-PLA_2 activity 1.5-fold and it was associated with a 3.5-fold reduction of macrophage adhesion ex vivo and with a 2.6-fold reduction in macrophage homing in vivo. These inhibitory effects were independent of the function of HDL as a cholesterol acceptor [110]. Furthermore, gene transfer of Lp-PLA_2 in apoE−/−/ mice increased plasma enzyme activity and this had as consequence a reduction in MDA-LDL autoantibodies and a decrease in the Lyso-PC/PC ratio in the β-VLDL fraction, which was associated with decreased deposition of oxidized lipoproteins and decreased accumulation of macrophages and smooth muscle cells in the arterial wall. This finally resulted in the inhibition of neointima formation induced by endothelial denudation and a reduction of spontaneous atherosclerosis [111]. In a subsequent study it was demonstrated that after a massive adenoviral overexpression of Lp-PLA_2 (76- to 140-fold increase of activity in plasma) in apoE−/− mice, Lp-PLA_2 was bound to all lipoprotein classes and protected them from Cu^{2+}-induced oxidation in vitro. Furthermore, it diminished the autoantibody titers against oxLDL in plasma of Lp-PLA_2-transfected mice and inhibited foam cell formation by enhancing HDL-induced cholesterol efflux from peritoneal macrophages in an in vitro cell model [112]. Consistent with the above results, local adenovirus-mediated *Lp-PLA_2* gene transfer at the site of injury resulted in a significant reduction in neointima formation in balloon-denuded aorta in cholesterol-fed rabbits [113]. Similarly, local expression of Lp-PLA_2 reduces accumulation of oxidized lipoproteins and inhibits inflammation; shear stress-induced thrombosis, and neointima formation in balloon-injured carotid arteries in normolipidemic rabbits [114]. It has been established that overexpression of the human apoA-I in mice raises HDL plasma levels and protects against atherosclerosis [115]. Moreover apoA-I overexpression is accompanied by a several-fold increment in HDL-Lp-PLA_2, which may enhance the anti-inflammatory and antioxidative potential of HDL [116]. Importantly, substitution of the Arg-123 — Tyr-166 central domain of apoA-I with the helical pair of apoA-II (Ser-12 — Ala-75) in apoE−/− mice, neither impaired HDL production nor was crucial for HDL-induced cholesterol influx. However this domain is required for increasing Lp-PLA_2 activity thus resulting in the reduction of oxidative stress in plasma and thereby, macrophage homing and early atherosclerosis in these animals [117]. Finally, HDL and HDL-Lp-PLA_2 may play a critical role in restoring the impaired migration of dendritic cells to lymphatic nodes and the suppressed immunologic priming, which is attributed to inhibitory signals generated by PAF or PAF-like molecules in dyslipidemic apoE−/− mice with atherosclerotic lesions [118]. Importantly, the impaired functionality of dendritic cells is restored by the intravenous injection of normal human HDL containing active Lp-PLA_2 but not by HDL particles containing either

chemically-inactivated Lp-PLA$_2$ with the serine-esterase inhibitor Pefabloc or lacking Lp-PLA$_2$ (HDL isolated from subjects bearing a missense mutation, Val279Phe substitution [118]. It should be emphasized that all the above studies have been conducted in animal models exhibiting reverse lipoprotein profile compared to humans (higher HDL than LDL levels), thus these results may not be relevant for the situation in humans. The antiatherogenic activities of HDL-Lp-PLA$_2$ are summarized in Fig. 2.

On a clinical basis of view, the decrease in the ratio of HDL-Lp-PLA$_2$ to total plasma enzyme or to LDL-Lp-PLA$_2$ observed in patients with dyslipidemias, may be useful as a potential marker of atherogenicity in these subjects [60,67,69]. Furthermore, recently published results showed that an increase in the ratio of LDL-Lp-PLA$_2$ to HDL-Lp-PLA$_2$ is associated with an increased incidence of paroxysmal atrial fibrilation and may be a marker for inflammation in these patients [119].

4.4. Lp(a)-Lp-PLA$_2$ as a scavenger of oxPL in human plasma

Lp(a) is an atherogenic lipoprotein and it is present in atherosclerotic but not in normal vessel walls. Lp(a) contributes to the formation of foam cells since it can be oxidized, aggregated or subjected to phospholipase A$_2$ modification and in this form it is taken up by scavenger receptors of macrophages [120]. In addition, Lp(a) influences the function of cells that play important roles in atherogenesis such as monocytes-macrophages and endothelial cells [121,122]. Lp(a) is enriched in Lp-PLA$_2$ [62–64]. The contribution of the Lp(a)-Lp-PLA$_2$ in the inflammatory processes in the artery wall could be significant, especially in patients exhibiting high levels of Lp(a) in plasma, since Lp(a) accumulates preferentially to LDL within lesions, and much of it is very tightly bound to lesion components [123,124]. Like LDL, Lp(a) is susceptible to oxidation, and oxidized Lp(a) is enriched in lyso-PC, due to hydrolysis of oxPL by Lp-PLA$_2$ [62]. Thus, the Lp(a)-Lp-PLA$_2$ may significantly influence the biological activities of oxidized Lp(a) in the artery wall, in which oxPL and lyso-PC are involved.

Importantly, results over the last years consistently report that oxPL in plasma are preferentially accumulated on Lp(a) [125]. Previous studies have demonstrated that oxPL can be directly bound to apo(a) forming covalent bonds with the active lysines of its kringle-V domain, although it is possible that there may be additional or alternative binding sites for oxPL on other kringles of apo(a). Furthermore, preliminary data suggest that additional oxPLs are present in the lipid phase of Lp(a) (Fig. 3). An important role in the accumulation of oxPL on Lp(a) may be also played by β2 glycoprotein I (β2GPI), which binds to apo(a), as well as to anionic phospholipids and oxPL [reviewed in 126] (Fig. 3).

In this regard more recent data showed that plasma levels of oxPL present on individual apoB-100 particles (oxPL/apoB levels), measured by the murine monoclonal antibody E06, are strongly correlated with plasma Lp(a) levels [127,128] and are preferentially bound by Lp(a) compared with other apoB-containing lipoproteins [125,127,128]. Furthermore, plasma oxPL/apoB levels are correlated with angiographically determined coronary artery disease and are elevated after acute coronary syndromes [126,127]. In fact, in most conditions studied thus far, >90% of circulating oxPL associated with apoB-100 particles, are actually present on Lp(a). These observations strongly support a physiological function of Lp(a) in preferentially binding oxPL compared to other apoB-100-containing particles. Importantly, the strongest correlation between oxPL/apoB and Lp(a) is observed in subjects with the smallest apo(a) isoforms [129]. One of the factors that could favor the sequestration of oxPL on small Lp(a) isoforms, could be the low catalytic efficiency of the Lp-PLA$_2$ associated with these isoforms [62]. The stronger association of oxPL with small Lp(a) isoforms may at least partially explain the enhanced atherogenicity of these isoforms as compared to large ones [130].

We had previously shown that the Lp(a) of patients with coronary artery disease, carries significantly less amount of Lp-PLA$_2$ mass that expresses lower catalytic efficiency compared to controls, a phenomenon which is not observed for the LDL-Lp-PLA$_2$ [63]. Importantly, the removal of apo(a) from the Lp(a) particle, resulted in a significant

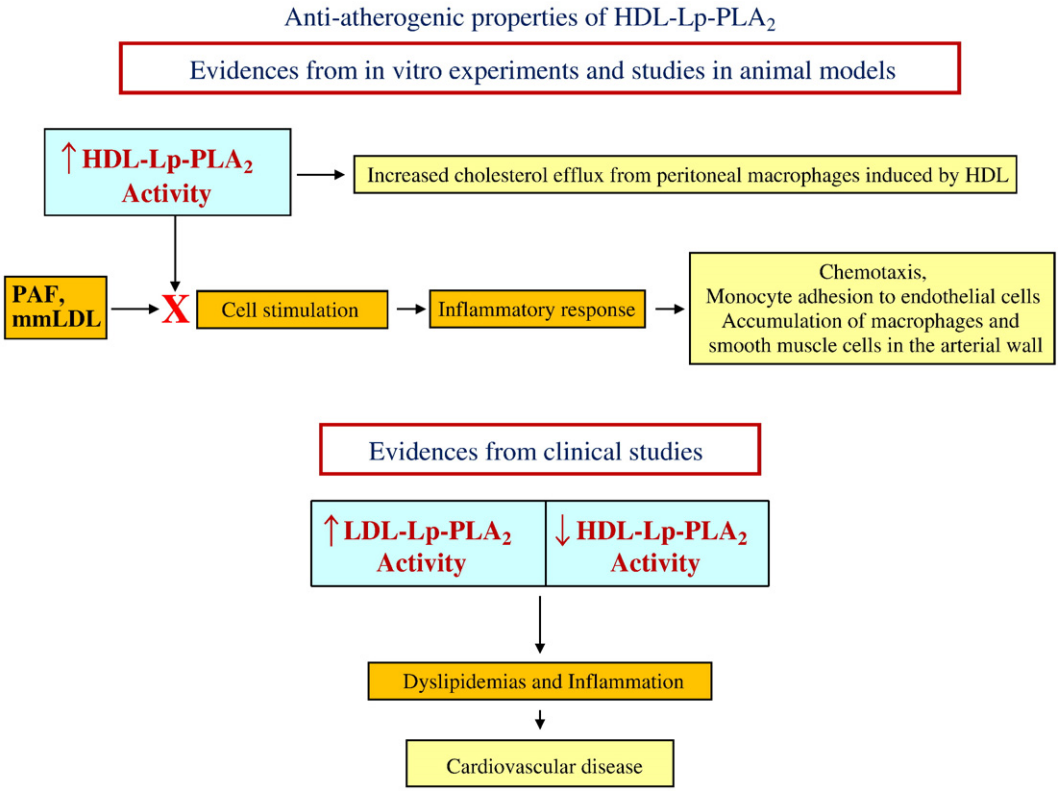

Fig. 2. Antiatherogenic effects of the HDL-associated Lp-PLA$_2$.

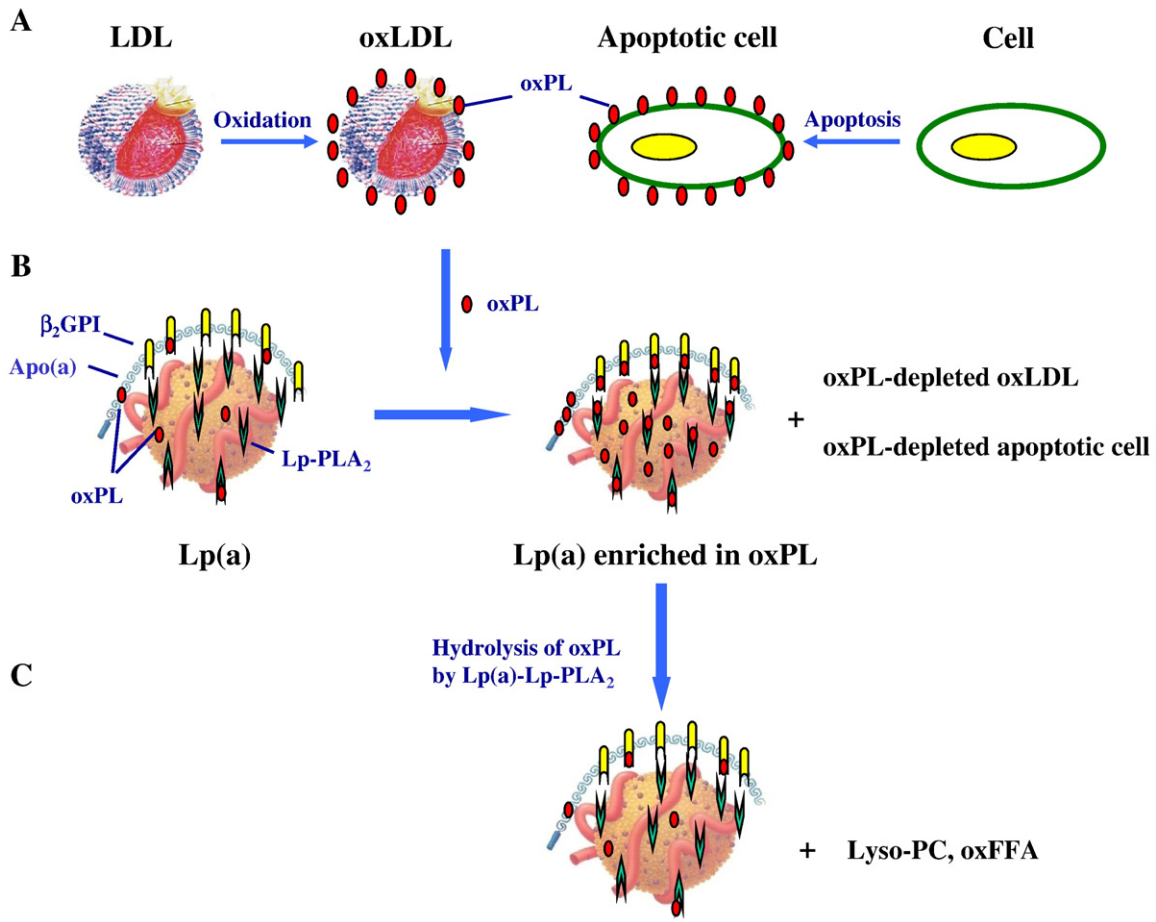

Fig. 3. Sequestration of oxidized phospholipids (oxPL) on Lp(a) and degradation by Lp(a)-associated Lp-PLA_2 (Lp(a)-Lp-PLA_2). (A) oxPL are formed during LDL oxidation or on cells submitted to oxidative stress or underwent apoptosis. (B) oxPL are then preferentially transferred to lipoprotein (a) [Lp(a)]. The mechanisms responsible for oxPL binding on Lp(a) are not fully determined yet, but may involve covalent binding to apolipoprotein(a) [apo(a)], binding to β-2 glycoprotein I (β_2GPI) which attaches to apo(a), and distribution in the lipid phase of Lp(a). (C) Once oxPLs are bound on Lp(a), they become accessible to endogenous Lp-PLA_2 and are degraded into lysophosphatidylcholine (lyso-PC) and oxidized free fatty acids (oxFFA). Modified from S. Tsimikas et al. [126].

increase in the Lp-PLA_2 catalytic efficiency, suggesting that the apo(a) moiety diminishes the enzyme activity expressed by Lp(a) in patients with coronary artery disease. Based on results from in vitro assays, we may hypothesize that a major factor responsible for the low catalytic efficiency of the Lp(a)-Lp-PLA_2 in patients with coronary artery disease, may be the sequestration of oxPL on the apo(a) moiety of Lp(a) [63].

The above observations suggest that oxPL formed on oxLDL or on cells submitted to oxidative stress or underwent apoptosis are released into the circulation from sources of cellular injury (such as plaque disruption and myocyte death) and are preferentially sequestered to Lp(a). These phospholipids are then hydrolyzed by the Lp(a)-Lp-PLA_2 into lyso-PC and oxFFA (Fig. 3). Thus, Lp(a) may act as a main transporter and scavenger of oxPL in the circulation. The pathophysiological significance of this Lp(a) function in respect to inflammation and atherosclerotic diseases needs further investigation [reviewed in 126].

4.5. PMPs-associated Lp-PLA_2 is an innocent bystander or an active participant in inflammation and atherosclerosis?

The presence of Lp-PLA_2 in circulating PMPs may be pathophysiologically important because PMPs express several protein receptors and ligands and contain biologically active lipids including PAF, which allows their interaction with various cells [131,132]. PAF especially plays an important role in cell-to-cell interactions observed in acute and chronic inflammation. Most signaling by PAF occurs between closely juxtaposed cells such as endothelial cells, neutrophils and monocytes [2,133]. Consequently, the PMP-associated Lp-PLA_2 may be important in regulating the activity of PMP-associated PAF in the juxtacrine signaling. It has been shown recently that PMPs are elevated in the circulation of patients with acute coronary syndromes [134,135]. In this context, we demonstrated that the balance between PAF production and secretion as well as Lp-PLA_2 secretion from platelets of patients undergoing coronary angioplasty is significantly altered before angioplasty as well as 48 h afterward [37]. This suggests that alterations in the balance between PAF and Lp-PLA_2 secreted by activated platelets may be of importance in coronary atherothrombosis and in the inflammatory response elicited during intracoronary injury induced by angioplasty [37]. Further studies are required to elucidate the possible role of PMPs in disseminating Lp-PLA_2 at sites of inflammation as well as in the arterial wall. In addition, the usefulness for quantitation of PMP-associated Lp-PLA_2 in plasma as a prognostic or diagnostic tool in atherosclerotic diseases should be investigated in large clinical studies.

5. Conclusions and perspectives

Although most of the data supporting the pro- or the antiatherogenic role of the LDL-Lp-PLA_2 is well established, the controversy still exists and several unanswered questions remain to be resolved. For example not all LDL particles contain Lp-PLA_2 [51], thus it remains to be established whether the biochemical and biological properties, and hence the atherogenic characteristics, of the LDL particles that carry

Lp-PLA_2 are different from those particles not carrying the enzyme. Furthermore, several studies have demonstrated that there is a wide spectrum of oxPL molecules having different structures and expressing either pro- or anti-inflammatory activities [24]. In addition, a number of substances derived from free oxidized fatty acids, like the F_2-isoprostanes, exert a variety of proatherogenic actions [136,137], whereas a recent study reported that cyclopentenone isoprostanes inhibit inflammatory responses in macrophages, and suggested that these molecules may serve as negative feedback regulators of inflammation [138]. Thus the substrate specificity of Lp-PLA_2 for the various types of oxPL, the equilibrium in the generation of proinflammatory versus anti-inflammatory oxPL and their downstream metabolic products during LDL oxidation, as well as the molar ratios of pro-/anti-inflammatory oxPL generated in vivo in the atherosclerotic plaque are important issues that remain to be defined. The above controversy becomes even more complex considering previous results showing that most of the inflammatory activities of lyso-PC are caused by the minute amount of PAF contained in this compound and are no longer manifested after the removal of the contaminating PAF [139]. Overall, the biochemistry and biology of the bioactive phospholipids generated from increased oxidant stress are complex and more studies are necessary in this area before any conclusion on the role of Lp-PLA_2 in atherosclerotic diseases can be made. Another important issue is that studies aimed at characterizing the expression levels of Lp-PLA_2 in human atherosclerotic plaques utilized immunological approaches from which it is not possible to predict to what extent enzymatic activity was preserved in this strongly oxidizing environment [107,108], considering that during LDL oxidation the Lp-PLA_2 activity is significantly reduced [31]. Thus further studies are necessary to evaluate the enzyme activity levels including the transesterification activity, which only recently was shown to exist in the atherosclerotic plaque [29]. Additional studies are also warranted to explore the potential causal role for Lp-PLA_2 in plaque progression and vulnerability.

Finally, on a clinical basis of view, further studies are required before a conclusion is reached as to whether Lp-PLA_2 is a risk factor for atherosclerotic diseases. In this setting, the results of clinical studies on the selective Lp-PLA_2 inhibitor darlapladib, being currently in progress, will definitely show whether Lp-PLA_2 is a causal mediator of atherosclerosis and whether this enzyme could be a target of therapy. Despite the unanswered questions on the role of Lp-PLA_2 in atherosclerosis, the determination of Lp-PLA_2 mass in plasma is currently recommended as a diagnostic test for vascular inflammation to better identify patients at high or very high cardiovascular disease risk who will benefit from intensification of lipid-modifying therapies [140].

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