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Inflammation, bioactive lipids and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A2, platelet activating factor-acetylhydrolase

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Abstract

It is well established that inflammation is an integral feature of atherosclerosis and of the cardiovascular diseases which it underlies. Oxidative stress is also recognized as a key actor in atherogenesis, in which it is closely associated with the inflammatory response and bioactive lipid formation. Several bioactive lipids have been identified in the atherosclerotic plaque, including the potent inflammatory mediator platelet activating factor (PAF), PAF-like lipids, oxidised phospholipids (oxPL) and lysophosphatidylcholine (lyso-PC). Recent evidence has established a central role of two phospholipases (PL) in atherogenesis, the non-pancreatic Type II secretory phospholipase A_2 ($sPLA_2$) and the lipoprotein-associated PLA_2—alternatively termed as PAF-acetylhydrolase (PAF-AH). $sPLA_2$ is calcium-dependent and hydrolyses the sn-2 acyl group of glycerophospholipids of lipoproteins and cell membranes to produce lyso-PC and free fatty acids. It is also implicated in isoprostane production from oxPL. $sPLA_2$ is an acute phase reactant, which is upregulated by inflammatory cytokines and may represent a new independent risk factor for coronary heart disease. In contrast to $sPLA_2$, PAF-AH is calcium-independent and is specific for short acyl groups at the sn-2 position of the phospholipid substrate and with the exception of PAF, can equally hydrolyze oxPL to generate lyso-PC and oxidized fatty acids. Thus PAF-AH plays a key role in the degradation of proinflammatory oxPL and in the generation of lyso-PC and oxidized fatty acids. PAF-AH equally can also hydrolyze short-chain diacylglycerols, triacylglycerols, and acetylated alkanols, and displays a PLA_1 activity. Whereas $sPLA_2$ may represent a new independent risk factor for coronary artery disease, the potential relevance of PAF-AH to atherosclerosis remains the subject of debate, and recent results suggest that the potential role of the LDL-associated PAF-AH in atherogenesis may be distinct to that of the HDL-associated enzyme. This review is focused on the main structural and catalytic features of plasma PAF-AH, on the association of the enzyme with distinct lipoprotein particle subspecies, on its cellular sources, and finally on the potential significance of this lipoprotein-associated PLA_2 in cardiovascular disease.

Keywords: Low density lipoprotein; High density lipoprotein; Lipoprotein subspecies; PAF-acetylhydrolase; Macrophage; Oxidised phospholipids; Small dense LDL; Coronary heart disease

1. Introduction

Over the past decade, it has become increasingly evident that inflammation is an integral feature of atherosclerosis and of the cardiovascular diseases which it underlies [1,2]. Indeed inflammatory processes contribute significantly to the initiation, progression and rupture of lipid-rich atherosclerotic plaques [1,2]. Plaque formation is frequently initiated at sites of predilection in the arterial tree such as the carotid bifurcation, at which enhanced intimal penetration and retention of atherogenic, cholesterol-rich lipoproteins such as LDL occur, and equally at which low oscillating shear stress—together with other factors, including hypercholesterolemia,—induce endothelial dysfunction [3]. Activation of the endothelium of the arterial intima is characterized by induction of oxidative stress, elevated endothelial permeability and expression of adhesion proteins for inflammatory cells [3,4]. Principal among the latter are monocytes which mature to monocyte-

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derived macrophages in the intima, and which undergo phenotype change with the expression of a spectrum of proinflammatory and prothrombotic factors, including metalloproteases, active oxygen species, phospholipases (PL), bioactive lipids, proinflammatory cytokines and tissue factor [5,6]. Indeed, there is abundant evidence to show that activated macrophages in the atherosclerotic plaque typically exhibit an inflammatory phenotype, as exemplified by their elevated expression of such factors in situ [1,2,4,5,7].

The intimal penetration and retention of atherogenic apoB-containing lipoproteins, including VLDL, VLDL remnants, IDL, LDL and Lp(a), is especially enhanced in atherogenic dyslipidemias such as hypercholesterolemia (Type IIA), mixed hyperlipidemia (Type IIB), and the dyslipidemia of Type II diabetes and the Metabolic syndrome, when circulating levels of atherogenic lipoproteins are elevated. The actions of oxygen free radicals, prooxidant enzymes (such as lipoxygenases) and PL lead to structural modification—and notably oxidation—of LDL, resulting in its uptake by macrophage scavenger receptors, with formation of lipid-laden foam cells, a characteristic component of atherosclerotic lesions [1–5,8,9]. Equally however, oxidized LDL itself possesses multiple proinflammatory properties [8,9], which in part reflect its content of oxidized, bioactive lipids and associated breakdown products [8–12].

2. Oxidative stress, bioactive lipid formation and PLA_2

Oxidative stress is now clearly recognized as a key actor in atherogenesis, in which it is closely associated with the inflammatory response and bioactive lipid formation. Several bioactive lipids have been identified in the plaque, including the highly potent inflammatory mediator platelet activating factor (PAF), PAF-like lipids, oxidised phospholipids (oxPL), oxidised cholesteryl esters (oxCE), oxidised free fatty acids, lysophosphatidylcholine (lyso-PC), oxysterols and isoprostanes [9–12]. Such bioactive lipids may have several origins, including secretion from activated macrophages (PAF, oxysterols), oxidation of lipid components of LDL (oxPL, oxCE, PAF-like lipids) and of cell membranes (oxPL, oxysterols), and PL-mediated cleavage of native and oxidized phospholipids (e.g. PAF, PAF-like lipids, lyso-PC, oxidized free fatty acids, isoprostanes). Significantly, bioactive lipids may exert both autocrine and paracrine effects, as exemplified by the activation of macrophage PAF receptors by PAF liberated from these cells.

Recent evidence has established a central role for the A_2 family of PL in inflammation and atherogenesis, and moreover suggests that both the Type II secretory phospholipase A_2 ($sPLA_2$) as well as the lipoprotein-associated PLA_2—alternatively termed PAF-acetylhydrolase (PAF-AH)—may be linked to coronary heart disease risk [12–17]. The non-pancreatic $sPLA_2$ is distinct from PAF-AH in that it is calcium-dependent, hydrolyses the sn-2-acyl group of glycerophospholipids of lipoproteins and cell membranes to produce lyso-PC and free fatty acids, shows wide tissue expression (hepatocytes, macrophages and arterial wall smooth muscle cells) and is implicated in isoprostane production from oxPL. Furthermore, $sPLA_2$ is an acute phase reactant, whose production is upregulated by inflammatory cytokines (IL1β, IL-6, TNFα) [13]. Consistent with the finding that plasma markers of inflammation (e.g. CRP, IL6) are elevated in subjects at high cardiovascular risk are the data of Kugiyama et al. [15], who observed significantly higher levels of plasma $sPLA_2$ in coronary artery disease patients as compared to controls, and in whom elevated $sPLA_2$ levels were a significant predictor of coronary events independent of other risk factors. Finally $sPLA_2$ present in arterial tissue may potentiate binding and retention of dense LDL [13], while transgenic mice which overexpress the enzyme show both an impaired antioxidant function of HDL and enhanced formation of bioactive oxidized phospholipids [14].

3. Human plasma PAF-acetylhydrolase or lipoprotein-associated PLA_2

Whereas it is now recognized that $sPLA_2$ may represent a new independent risk factor for coronary artery disease, the potential relevance of PAF-AH to atherosclerosis remains the subject of debate. This review is therefore focused on the main structural and catalytic features of circulating PAF-AH, on the association of the enzyme with distinct lipoprotein particle subspecies, on its cellular sources and the effect of proinflammatory mediators on enzyme expression, and finally on the potential significance of this lipoprotein-associated PLA_2 on cardiovascular disease.

4. Structural and catalytic properties

The major features of the structure, catalytic properties and plasma transport of PAF-AH are summarized in Table 1. The presence of an enzyme in human plasma that catalyzes the hydrolysis of the sn-2 ester bond of the potent proinflammatory phospholipid, PAF,—and thus attenuates its bioactivity—was first demonstrated by Farr et al. in 1980 [18]. Subsequently this enzyme was named as PAF-AH (EC 3.1.1.47) and found to be associated with plasma lipoproteins [19–21]. Plasma PAF-AH is categorized in the subfamily VIIA of PLA_2, and its activity does not require calcium. Unlike other PLA_2, PAF-AH is specific for short acyl groups (Cn <

Table 1
Major features of the structure, catalytic properties and plasma transport of PAF-AH

(1)	45.4-kDa monomeric protein.
(2)	Contains N-linked heterogenous sugar chain(s), 9 kDa, involving sialic acid.
(3)	The cDNA encodes a 441-amino acid protein containing a secretion signal sequence (Met-1–Ala-17).
(4)	The catalytic site contains the Gly-His-Ser-Phe-Gly consensus sequence characteristic of lipases and esterases.
(5)	Ser-273, Asp-296 and His-351 arc essential for catalytic activity consistent with an α/β hydrolase conformation.
(6)	Expresses Ca^{2+}—independent PLA_2 activity towards PAF and oxidized phospholipids.
(7)	Expresses lipase, transacetylase and PLA1 activities.
(8)	Enzyme sources: cells of hematopoietic origin (monocytes–macrophages, hepatic kupffer cells, mast cells, platelets).
(9)	The gene is located at chromosomal region 6p12–21.1, and comprises 12 exons.
(10)	Enzyme expression is primarily regulated by the differentiation state of the cell and by proinflammatory mediators.
(11)	Plasma transport; 80–85% LDL (primarily small-dense LDL); 15–20% HDL.
(12)	PAF-AH binds directly to –COOH terminal of LDL-Apo B100 (PAF-AH residues: Tyr 205, Tryp 115, Leu 116).

6) at the sn-2 position of the phospholipid substrate [19,22]. Plasma PAF-AH is enzymatically active, and its highly restricted substrate specificity is essential to prevent the continuous hydrolysis of the phospholipids of lipoproteins and cell membranes [23]. With the exception of PAF, PAF-AH can also effectively hydrolyze oxidized phospholipids produced by peroxidation of phosphatidylcholines containing an sn-2 polyunsaturated fatty acyl residue [24–26] (Fig. 1). Such oxidized phospholipids are formed during the oxidative modification of LDL and play key roles in several aspects of atherogenesis [27]. Early studies had shown that PAF-AH is an interfacial enzyme [19] however, more recent work has revealed that PAF-AH access its substrates only from the aqueous phase, thus this enzyme may hydrolyze other lipid esters that are partially soluble in the aqueous phase [28]. Indeed, with the exception of its PLA_2 activity, PAF-AH equally can hydrolyze short-chain diacylglycerols, triacylglycerols, and acetylated alkanols, and also displays a PLA1, activity. Consequently PAF-AH possesses a broad substrate specificity toward lipid esters containing short acyl chains. Since oxidative damage occurs not only in phospholipids but also in various types of compounds containing unsaturated bonds, this enzyme may play an important antioxidative scavenger role [29]. In addition to the lipase and esterase activities of plasma PAF-AH, this enzyme also exhibits a transacetylase activity. Indeed semipurified PAF-AH from human plasma was able to transfer the acetate group from PAF to 1-acyl-sn-glycero-3-phosphocholine (lyso-PC) [30]. In this context, it is relevant that we demonstrated the presence of a transacetylase activity in LDL that transfers acetate as well as short-chain fatty acids from PAF and its ether- and ester-linked analogues to ether/ester-linked lysophospholipids. The substrate specificity of the donor molecules was decreased by increasing the chain length of the acyl moiety in the sn-2 position, whereas the ether linkage in the sn-1 position of the glycerol moiety was 30% more effective than the ester bond. The two acceptor molecules tested, lyso-PAF and lyso-PC, showed similar specificity. Such activity was attributed to the LDL-associated PAF-AH. Furthermore, similar transacetylase activity was exhibited by recombinant human plasma PAF-AH [31].

Plasma PAF-AH is resistant to proteolysis and is unaffected by reagents that target sulfhydryl or histidyl residues, but it is markedly inhibited by serine esterase inhibitors such as diisopropylfluorophosphate and Pefabloc [32,33].

The cloning of a cDNA encoding the human plasma PAF-AH was reported by Tjoelker et al in 1995 [34] and subsequently confirmed by other groups [35]. The cDNA contains an open reading frame predicted to encode a 441-amino acid protein which is cleaved between Lys-41 and Ile-42 to generate a mature enzyme with a calculated molecular mass of 45.4 kDa. The first 17 residues (Met-1 to Ala-17) are hydrophobic and form a typical secretion signal sequence [34]. The next 24 predicted residues (Val-18 to Lys-41) were not found in the protein purified from plasma. In this context, three different N-termini have been noted in purified plasma PAF-AH and it is uncertain whether this finding represents a purification artifact or whether there is heterogeneity in the plasma enzyme [36]. The primary structure of PAF-AH is unique with the exception of the Gly-His-Ser-Phe-Gly consensus sequence in the catalytic site of the enzyme, which is similar to that found in serine esterases and lipases. With the use of site-directed mutagenesis, it was shown that Ser-273 of the Gly-His-Ser-Phe-Gly motif as well as Asp296 and His-351 are essential for catalytic activity [36]. The presence of the Gly-His-Ser-Phe-Gly motif in PAF-AH is consistent with the sensitivity of the enzyme to serine esterase inhibitors. The linear orientation and spacing of Ser-273, Asp-296 and His-351 are consistent with the α/β hydrolase conformation which is supported by preliminary structural modeling studies (reviewed in [37]). These features indicate that PAF-AH is structurally and mechanistically different from PLA_2, consistent with the recently proposed broad substrate specificity of this latter enzyme. PAF-AH in plasma is N-glycosylated and contains about 9 kDa of a heterogenous asparagine–conjugated sugar chain(s), involving sialic acid [35,38]. According to the primary structure of the enzyme, such glycosylation could occur at two asparagine residues, i.e. at Asp-423 and Asp-433.

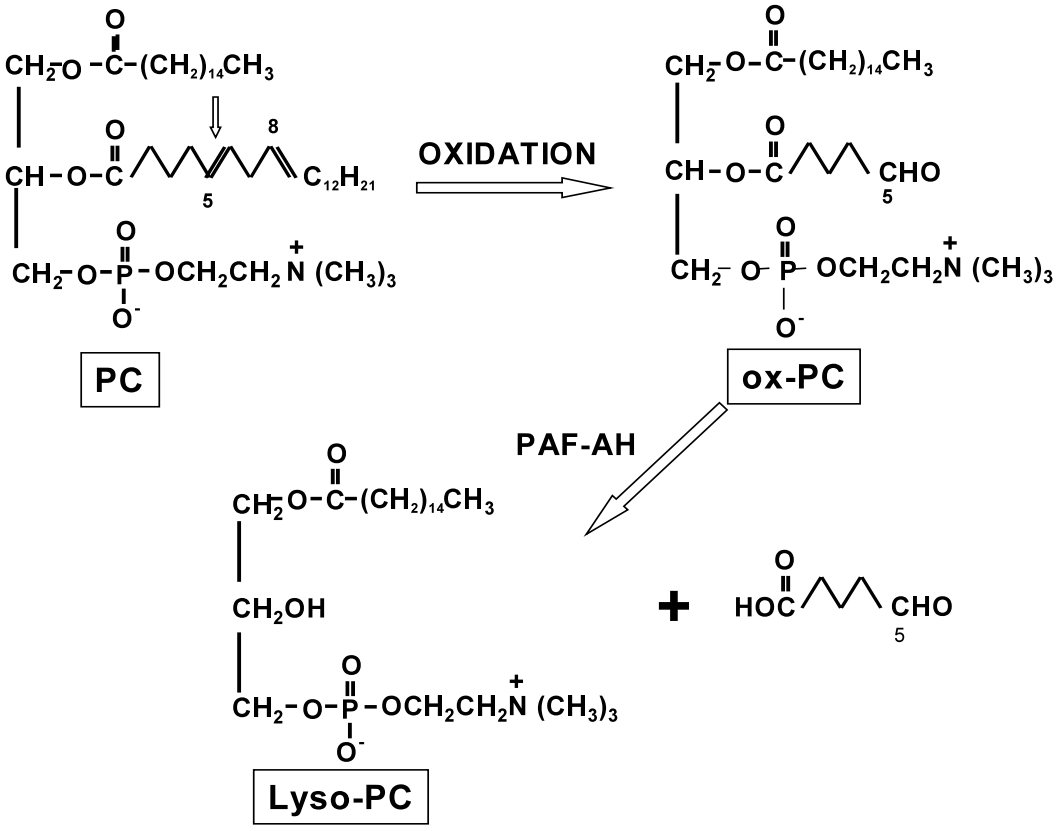

Fig. 1. Hydrolysis of oxidized phospholipids by PAF-AH.

5. Association with plasma lipoprotein particle subspecies

In human plasma, PAF-AH is primarily associated with LDL particles, whereas a small proportion ($< 20\%$ of total enzyme activity) is associated with HDL (Table 1). Within these lipoprotein pools, it appears that the enzyme preferentially associates with small dense LDL and with the very high density lipoprotein-1 subfraction [39]. It has been reported that during plasma ultracentrifugation, a minor proportion of the LDL-associated PAF-AH dissociates from the particle, suggesting the presence of dissociable and nondissociable forms of the enzyme on LDL. Interestingly, a greater proportion of the dissociable form of PAF-AH was found in subjects exhibiting LDL pattern B (primarily small dense LDL), compared with subjects exhibiting the LDL pattern A (primarily large buoyant LDL) [40]. However, the potential significance of the dissociable form of PAF-AH in vivo remains to be determined. The distribution of PAF-AH between LDL and HDL can be influenced by the presence of Lp(a) when plasma levels of this lipoprotein exceed 30 mg/dl [41]. Lipoprotein(a) contains several-fold greater PAF-AH activity compared with LDL when assayed at equimolar protein concentrations [42]. Furthermore, apo (a) isoform size significantly influences the kinetic constants of the enzyme associated with Lp(a), suggesting that apo (a) influences association of the enzyme with Lp(a), although this apolipoprotein does not bind PAF-AH itself [41]. Thus a major role in the association of PAF-AH with LDL and Lp(a), is played by the apo B-100 moiety. Indeed, using site-directed mutagenesis, Stafforini et al. [43] demonstrated that PAF-AH interacts directly with apo B-100, and that the carboxyl terminus of this apolipoprotein is required for such interaction. On the PAF-AH side, residues Try-115, Leu-116 and Tyr-205 are critical for association of the enzyme with LDL (Table 1) [43]. Consistent with this finding, Try-115 and Leu-116 residues are not conserved in mouse PAF-AH, and consequently the mouse enzyme is unable to associate with human LDL. However, substitution of the human Try-115 and Leu-116 residues for the corresponding mouse residues enables mouse PAF-AH to bind LDL [36,43].

In contrast to the well-studied association between LDL and PAF-AH, there is a paucity of data concerning the association of this enzyme with HDL. Recently we provided evidence that removal of the carbohydrate content of the macrophage-derived PAF-AH (which represents one of the major sources of the enzyme pool present 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 PAF-AH with LDL versus

HDL could be the degree of enzyme glycosylation [44]. Recently, it has been shown that human plasma paraoxonase 1 (PON1), an enzyme exclusively associated with HDL, exhibits PAF-AH activity in addition to its activities toward paraoxon and phenylacetate [45]. In this context, it has been observed that PAF-AH protein is lacking in HDL, suggesting that the HDL-associated PAF-AH activity is due to PON1 [45].

Another enzyme that could potentially contribute to HDL-associated PAF-AH activity is lecithin-cholesterol acyltransferase (LCAT), a plasma enzyme that circulates mainly associated with HDL and is responsible for the formation of most of the cholesterol esters present in human plasma [46]. Although the major function of LCAT involves cholesterol esterification, several studies have shown that LCAT can also hydrolyze PAF and oxidized species of phosphatidylcholine; thus LCAT exhibits PAF-AH-like activity [30,47]. However, unlike the activity of PAF-AH, the PAF-AH-like activity of LCAT is not manifested in normal plasma, because it is strongly inhibited by free cholesterol which binds to LCAT [48]. However, when plasma is subjected to oxidative stress, the free cholesterol esterification activity of LCAT is rapidly lost while the PAF-AH-like activity is stimulated [49,50]. This phenomenon has been attributed to the loss of the cholesterol binding capacity of LCAT induced by lipid peroxidation products. In conclusion, although LDL-associated PAF-AH activity seems to be exclusively due to PAF-AH protein, HDL-associated PAF-AH activity may derive from PAF-AH-like activities expressed by different enzymes associated with this lipoprotein (Table 2). The relative contribution of the PAF-AH protein itself to this activity remains to be established.

Table 2
Properties and antiatherogenic role of the HDL-associated PAF-AH activity

(1) Represents a pool of similar catalytic activities expressed by PAF-AH, LCAT, and PON1
(2) The glycosylation of PAF-AH is a major determinant of the enzyme association with HDL
(3) Contributes to the HDL-induced protection of LDL from oxidation.
(4) Plays an important role in the HDL-mediated inhibition of cell stimulation induced by oxidized LDL in vitro.
(5) Adenoviral transfer of human plasma PAF-AH gene in apo E−/− mice increases HDL-associated enzyme activity and reduces atherosclerosis.
(6) The decrease in the ratio of plasma HDL-PAF-AH activity to LDL-cholesterol levels in primary hypercholesterolemia is proportional to the severity of hypercholesterolemia and may represent a marker of atherogenicity.

6. Cellular sources of human plasma PAF-AH

Early studies demonstrated that peripheral blood monocyte-derived macrophages [51], and the human hepatocarcinoma cell line HepG2 [52,53] secrete the plasma form of PAF-AH. Other cell types that secrete this enzyme are neutrophils, differentiated HL-60 cells, activated bone marrow-derived mast cells, and activated platelets [54–58]. The PAF-AH mRNA has been detected in differentiated macrophages, as well as in thymus tonsils and human placenta, but not in liver cells and monocytes. Such a cellular location of PAF-AH mRNA is consistent with a macrophage origin of the enzyme [34].

Levels of PAF-AH mRNA were essentially undetectable in normal rat liver. However, 24 h after treatment of animals with lipopolysaccharide (LPS), a 20-fold increase in the abundance of PAF-AH mRNA was observed, but which was restricted to Kupffer cells [59]. Furthermore, Kupffer cells in culture secrete 20–25 times more PAF-AH activity as compared with cultured hepatocytes [60]. The above data suggest that hepatocytes do not appear to significantly contribute to the plasma pool of PAF-AH. In contrast, these cells may represent the major source of PAF-AH found in bile [60,61]. By contrast, recent studies have revealed that cells of hematopoietic origin constitute the primary source of circulating PAF-AH. Thus, the mRNA of plasma PAF-AH has been detected in lipid-laden macrophages in human atherosclerotic lesions [62]. Furthermore, Asano et al. [63] reported that when a Japanese patient with normal plasma PAF-AH activity received an allogenic bone marrow transplant from a donor who was homozygous for the PAF-AH mutation V279F, (which results in complete PAF-AH inactivation), transplantation resulted in lack of PAF-AH activity in the recipient's serum. However, this phenomenon was not observed in patients who received bone marrow from donors with normal serum PAF-AH levels or from heterozygotes for the above PAF-AH mutation, as the recipients displayed normal or half normal serum PAF-AH activity, respectively [63]. Overall, the above studies strongly suggest that plasma PAF-AH derives from cells of hematopoietic origin (Table 1). Our recent finding that the glycosylation of PAF-AH secreted from human blood monocyte-derived macrophages resembles that of the plasma enzyme is consistent with this possibility [44]. Since cells of hematopoietic origin do not secrete lipoproteins, secretion of PAF-AH occurs independently of the secretion of lipoprotein particles; the enzyme subsequently associates with these particles in plasma [23]. Consistent with this hypothesis, PAF-AH activity in plasma of individuals with HDL deficiency (Tangier disease) is higher than that of normal subjects [64], whereas individuals with abetalipoproteinemia have normal or slightly subnormal PAF-AH

activity [65,66]. Although the secretion of lipoproteins does not seem to affect PAF-AH secretion, the lipoprotein environment in which the enzyme is subsequently inserted significantly influences the catalytic properties of the enzyme [65].

7. Effect of proinflammatory mediators on PAF-AH expression

The cellular expression of plasma PAF-AH is regulated by various factors, including the differentiation state of the cell and the degree of activation by proinflammatory mediators (Table 1). Most studies have been focused on monocyte-derived macrophages, tissue macrophages, and HL-60 cells. Human peripheral blood monocytes do not express PAF-AH, however upon differentiation into macrophages in vitro, PAF-AH mRNA becomes detectable and enzyme activity is secreted into the culture medium [44,51]. Consistent with this finding, macrophages in human atherosclerotic lesions express plasma PAF-AH [62]. However, other types of tissue macrophages such as rat Kupffer cells contain undetectable levels of PAF-AH mRNA, which is upregulated during in vitro culture [59]. Consequently, the dependence of PAF-AH expression on cellular differentiation state does not always reflect the situation in all types of tissue macrophages, suggesting that other factors may also play important roles. Most of the proinflammatory mediators (LPS, TNF-α, IL-1, IL-8, and IFN-γ) inhibit PAF-AH expression by various types of macrophages in vitro [54,59,67–70], whereas PAF stimulates enzyme expression in differentiated monocyte-derived macrophages [68]. Consistent with the reduction in PAF-AH expression and secretion by macrophages induced by proinflammatory mediators are the results of our study in patients with juvenile rheumatoid arthritis, in which patients with active disease presented with lower plasma PAF-AH activity as compared to inactive disease patients and controls [71]. Furthermore, enzyme activity was negatively correlated with plasma CRP levels [71]. Reduced PAF-AH activity has equally been observed in other inflammatory diseases such as systemic lupus erythematosus [72], sepsis [73] and Crohn's disease [74]. In contrast to the results of the above in vitro and clinical studies, in vivo administration of proinflammatory mediators (LPS, TNF-α, IL-1) in experimental animals has revealed a significant increase in plasma PAF-AH activity [59,75]. Such contrasting results remain to be explained. Elevation in PAF-AH activity induced by inflammatory mediators in vivo may however occur via their effects on lipoprotein metabolism [76,77]. Indeed, although the secretion of lipoproteins does not appear to influence PAF-AH secretion, their intravascular metabolism and consequently their rate of removal from the circulation is a key factor influencing levels of PAF-AH activity in plasma.

The promoter of the gene coding for the plasma form of PAF-AH contains seven MS2 and eleven STAT binding consensus sequences [68]. The multiple MS2 binding sites indicate that expression of the PAF-AH gene is under tight differentiation control, whereas the STAT consensus sequences may mediate the effects of inflammatory mediators on PAF-AH gene expression. Future studies of the regulation of PAF-AH gene expression will clarify the effects of proinflammatory mediators on PAF-AH expression.

8. Role of plasma PAF-AH in atherosclerosis

PAF-AH may play a significant role in atherogenesis and cardiovascular disease due to its role in the metabolism of bioactive lipids such as PAF and oxidized phospholipids. PAF activates leukocytes and platelets and enhances leukocyte adhesion to the vessel wall. Furthermore, PAF is a vasoactive mediator which may be synthesized locally at the site of endothelial injury during thrombosis. Equally PAF 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 Refs. [78,79]). 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 [80–82]. Consequently, by degrading such phospholipids, PAF-AH could act as a potent antiatherogenic enzyme. Indeed various studies in vitro support such a role. Thus it has been shown that PAF-AH by degrading oxidized phospholipids inhibits the oxidative modification of the apoB moiety of LDL, suggesting that intact but not hydrolyzed phospholipids are required for apoB modification [83]. In contrast PAF-AH does not prevent the lipid peroxidation of LDL [83,84]. Furthermore, PAF-AH inhibits several proatherogenic activities of oxidized LDL in vitro in which oxidized phospholipids are involved [85]. Interestingly, mildly oxidized apoB-containing lipoproteins depleted of PAF-AH activity exhibit increased stimulation of monocyte chemotaxis and adhesion compared with non-PAF-AH depleted lipoproteins, an activity which was blocked by PAF-receptor antagonists [86]. Furthermore, minimally modified lipoproteins from Japanese subjects with a deficiency of plasma PAF-AH activity were markedly more bioactive compared with lipoproteins from control subjects with normal plasma PAF-AH [86].

During oxidation, LDL-associated PAF-AH activity is progressively lost [80,87], suggesting that such inactivation may facilitate the accumulation of oxidized

phospholipids on LDL. The important role of PAF-AH in preventing the accumulation of biological active oxidized phospholipids on LDL during oxidation has been further supported by the fact that during oxidation PAF is formed and accumulates in PAF-AH, poor, large and intermediate LDL particles in much higher amounts as compared with small-dense LDL, as a result of its enrichment in PAF-AH activity [88].

In contrast to the above hypothesis involving an antioxidant, anti-inflammatory and antiatherogenic role for LDL-associated PAF-AH, other studies have suggested that it can be a proinflammatory and proatherogenic enzyme. This is supported by the observation that during the hydrolysis of oxidized phospholipids PAF-AH generates lyso-PC [24], a phospholipid which participates in several aspects of plaque formation [27]. In addition to lyso-PC, oxidized fatty acids exhibiting proatherogenic activities are liberated during hydrolysis of oxidized phospholipids by PAF-AH [89]. Moreover, the enrichment of dense LDL with PAF-AH has as a consequence the enhanced production of lyso-PC during oxidation of this particle compared to large-light ones (the large, light LDL subspecies), in both normolipidemic and hypercholesterolemic patients [90,91]. Thus under the action of PAF-AH the dense LDL particles are enriched with proatherogenic lyso-PC. We have also shown recently that in the presence of exogenous lyso-PAF, dense LDL-associated PAF-AH can catalyze a transesterification reaction leading to transformation of inactive lyso-PAF to proinflammatory PAF [31]. Consistent with a proatherogenic role for LDL-associated PAF-AH are recent in vitro data showing that inhibition of endogenous PAF-AH prior to LDL oxidation by serine esterase inhibitors or by the specific PAF-AH inhibitor SB222657 inhibited the rise in lyso-PC levels in oxidized LDL, and diminished the capacity of oxidized LDL to induce apoptosis and toxicity in human macrophages [92]. Overall, from the above controversial results, it is not evident as to whether the equilibrium between the degradation and generation of proatherogenic phospholipids observed in in vitro studies of isolated LDL may favor the antiatherogenic or proatherogenic role of LDL-associated PAF-AH. The fact that PAF and oxidized phospholipids are active at concentrations several fold lower as compared to those of lyso-PC rather favors the antiatherogenic role of PAF-AH. However the molar ratios and the equilibrium between the above phospholipids in vivo in the atherosclerotic plaque, in which LDL coexists with HDL as well as other factors that could influence oxidative stress in situ, remain to be defined.

Contrasting findings have been made in vivo concerning plasma PAF-AH activity, which mainly reflects LDL-associated PAF-AH. Plasma PAF-AH activity increases gradually with age, and exhibits an up to fivefold variation in healthy adult populations [93–96]. About 60% of this variation can be accounted for by genetic factors [96]. Plasma LDL concentration exerts major influence on PAF-AH activity. Indeed several studies have demonstrated a strong correlation between enzyme activity and plasma LDL-cholesterol or apoB levels [96,97]. We recently showed that among LDL subfractions, plasma PAF-AH activity is positively correlated only with dense LDL subfractions, thus further supporting the preferential association of PAF-AH with small, dense LDL particles [97]. Since the production and secretion of PAF-AH and LDL occur independently, the influence of LDL levels on plasma PAF-AH levels suggests that the key factor which determines plasma levels of PAF-AH is the rate of removal of LDL from the circulation [96,97]. Consistent with this notion is the observation that PAF-AH-rich, small dense LDL particles are more slowly removed from the circulation as compared to the larger lighter ones, due to their reduced binding to the cellular LDL receptor [98].

Recently we have shown that plasma PAF-AH activity and that specifically associated with LDL particles in patients with primary hypercholesterolemia without clinical evidence of coronary artery disease preferentially increases relative to that associated with HDL, in parallel with increase in the severity of hypercholesterolemia. Indeed, the highest levels are seen in homozygous familial hypercholesterolemia [97], in which the lowest rate of LDL clearance is observed among all forms of primary hypercholesterolemia [99]. The dependence of plasma PAF-AH activity on LDL clearance rate is further supported by the effect of lipid lowering therapy with HMG-CoA reductase inhibitors (statins) in patients with hyperlipidemia. Statins enhance LDL clearance from plasma via upregulation of hepatic LDL receptors and increase of LDL FCR [100]. We recently showed that atorvastatin therapy in patients with primary hypercholesterolemia, as well as in those with combined hyperlipidemia significantly reduces total plasma- and LDL-associated PAF-AH activity; this effect occurred in parallel with reduction in plasma LDL-cholesterol levels [101]. This observation is also supported by the positive correlation observed between the reduction of plasma LDL cholesterol levels and that of plasma PAF-AH activity. Atorvastatin therapy did not affect HDL-associated PAF-AH activity. A similar effect on plasma PAF-AH activity was observed for lovastatin therapy in non-insulin dependent diabetes mellitus and in obesity [102], as well as in hypercholesterolemic patients [96]. The reduction in plasma enzyme activity by atorvastatin cannot however be attributed to a decrease in PAF-AH secretion from its main cellular sources, since atorvastatin did not reduce PAF-AH secretion from macrophages in vitro [101].

Although rates of LDL clearance could account for both the higher plasma and LDL-associated PAF-AH

activity observed in hypercholesterolemia, the possibility that elevated circulating LDL particle numbers in such patients could enhance PAF-AH secretion from its cellular sources cannot be excluded. Indeed, it has been shown that PAF-AH secretion from macrophages is enhanced when serum is present [51] and LDL may represent one of the serum constituents responsible for this effect (Tselepis et al., unpublished observations). Consequently, high LDL plasma levels, typical of hypercholesterolemic patients, may influence secretion of PAF-AH from macrophages.

It is well documented that patients with primary hypercholesterolemia exhibit premature atherosclerosis, mainly as a result of high plasma LDL levels [100]. The elevated plasma PAF-AH activity in these patients is indicative of the high plasma LDL levels and thus plasma PAF-AH may be considered as a marker of atherogenesis and cardiovascular risk. However, from a pathophysiological basis of view, it is not known whether the increased PAF-AH activity promotes atherogenesis or whether it represents a defense mechanism against enhanced plaque formation and an inflammatory response to bioactive lipids in these patients. Data from clinical studies have revealed inconsistent findings regarding plasma levels of PAF-AH activity in atherosclerotic disease. Thus, some studies have shown an elevation [103,104], others have reported lower enzyme activity as compared to controls [105–107], whereas some studies have failed to detect any difference between patients with atherosclerotic disease and controls [108,109]. Recent results from the WOSCOPS study suggest that plasma levels of PAF-AH mass, which is proportional to its activity and mainly reflects the LDL-associated enzyme, represent an independent risk factor for coronary artery disease [16]. In contrast, a recent prospective nested case-control study in apparently healthy middle-aged women who participated in the Women's Health Study (WHS), suggested that plasma PAF-AH mass is not a strong predictor of future cardiovascular risk over a mean follow-up of 3 years [110].

A valuable molecular tool in the evaluation of the role of plasma PAF-AH in cardiovascular disease as well as in other diseases is provided by the $G^{994} \rightarrow T$ missense mutation in exon 9 of the plasma PAF-AH gene (located in chromosome 6p12 21.1 and comprised of 12 exons [111]. This mutation is located within the catalytic domain of the enzyme and results in a nonconservative valine to phenylalanine transition at position 279 (V279F) and in complete PAF-AH inactivation. This mutant allele has been detected in members of 14 Japanese families and is present in 27% as heterozygotes and 4% as homozygotes. The $G^{994} \rightarrow T$ mutation is an independent risk factor for stroke [112] and coronary artery disease in Japanese men, especially in low-risk individuals who lack conventional risk factors [113]. Furthermore more recent studies showed that this mutation may be one of the genetic determinants of atherosclerotic disease in the Japanese population [114]. Overall, in vivo data on the potential role of total plasma- and LDL-associated PAF-AH in atherosclerotic disease does not allow us to draw definitive conclusions, although most studies indicate that plasma PAF-AH activity is a marker of plasma LDL levels and even of dense LDL levels.

In contrast to the controversy regarding the potential role of LDL-associated PAF-AH in atherosclerosis, several lines of evidence suggest that HDL-associated PAF-AH activity, although much lower than that in LDL may contribute substantially to (1) protection of LDL from oxidation, and (2) to the HDL-mediated inhibition of cell stimulation induced by oxidized LDL (Table 2) (reviewed in [115,116]). In human plasma HDL, such activity may represent a pool of similar catalytic activities expressed by three different enzymes, PAF-AH, LCAT [35] and PON1 [14]. In a previous study however, it was reported that there is no PAF-AH protein on HDL, suggesting that PAF-AH protein may not contribute to HDL-associated PAF-AH activity [14].

It was shown recently that PAF-AH exhibits antiatherogenic activity in apoE−/− mice, an especially pertinent finding as PAF-AH in murine plasma is exclusively associated with HDL [117]. Adenoviral transfer of the human plasma PAF-AH gene in these animals increased PAF-AH activity 1.5-fold and was associated with a 3.5-fold reduction of βVLDL-induced ex vivo macrophage adhesion 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 [118]. Furthermore, gene transfer of PAF-AH in apoE−/− mice inhibited injury-induced neointima formation and spontaneous atherosclerosis [119]. This approach, which distinguishes the role of PAF-AH activity associated with atherogenic LDL from that associated with antiatherogenic HDL, could at least partially explain the discrepancy between the results of the WOSCOPS study (PAF-AH is an independent predictor for coronary artery disease), and those in the Japanese population with the $G^{994} \rightarrow T$ mutation (loss of PAF-AH activity is a genetic determinant for atherosclerotic disease). Thus, the loss in enzyme activity due to the $G^{994} \rightarrow T$ mutation probably reflects not only the LDL-associated enzyme but also HDL-associated PAF-AH activity, since no activity is found in the plasma of this population [111–114]. Thus the absence of PAF-AH activity on HDL rather than on LDL may represent the major determinant for atherogenesis in this population. We recently demonstrated that patients with primary hypercholesterolemia exhibit an alteration in the relative distribution of PAF-AH among LDL and HDL resulting in a decrease in the ratio of HDL PAF-

AH to plasma PAF-AH (or to LDL-cholesterol levels), which is proportional to the severity of the hypercholesterolemia [97]. This ratio may be useful as a potential marker of atherogenicity in subjects with primary hypercholesterolemia and is consistent with our previously published data on patients with unstable angina [109].

9. Conclusions and perspectives

In light of this review, recent data have considerably clarified the relationship of the two distinct plasma forms of PAF-AH, i.e. LDL-associated PAF-AH and the HDL-associated enzyme, to the inflammatory dimension of atherosclerosis. Thus, abundant evidence indicates that PAF-AH or PAF-AH-like activity associated with HDL particles plays a predominantly antiatherogenic, anti-inflammatory role through its reduction of monocyte adhesion to endothelium, its ability to abrogate the biological activity of minimally modified LDL, and finally its capacity to attenuate phospholipid oxidation and induce reduction in lesion volume via a decrease in macrophage homing in animal models. By contrast, the role of LDL-associated PAF-AH remains controversial, possibly as a result of difficulty in dissecting the actions of the enzyme itself from that of the atherogenic dense LDL particle on which it is predominantly transported. Ultimately however, this question should be resolved by careful comparison of the proinflammatory actions of the oxidised phospholipid substrates of the LDL-associated enzyme versus those of the bioactive lipids which it liberates from the lipoprotein particle.

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