The emerging roles of PAF acetylhydrolase

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Abstract Platelet-activating factor (PAF), a phospholipid autacoid with potent effects throughout the innate immune system, is selectively degraded by two small families of PAF acetylhydrolases (PAF-AHs). These Ca^{2+}-independent phospholipases A_2 display remarkable specificity for the length of the *sn*-2 residue, but this selectivity is lost as the residue gains oxygen functions. Two of the PAF-AHs therefore are specific oxidized phospholipid phospholipases that reduce inflammation, but also remove oxidatively truncated phospholipids that induce apoptosis.▮ The roles of these enzymes are manifold, and their separate and combined functions are now being addressed in model systems and clinical studies—McIntyre, T. M., S. M. Prescott, and D. M. Stafforini. **The emerging roles of PAF acetylhydrolase.** *J. Lipid Res.* 2009. S255–S259.

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PAF ACETYLHYDROLASES: UNIQUE PHOSPHOLIPASES A_2

The phospholipid autacoid platelet-activating factor (PAF; 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is remarkably potent; it activates cells at picomolar concentrations; and its single receptor (1) is expressed by numerous cells, including all those of the innate immune system, making it an important intercellular mediator. PAF acetylhydrolases (PAF-AHs) are a small family of related phospholipases A_2 that hydrolyze the *sn*-2 acetyl residue of this inflammatory mediator, thereby inactivating it (2). A distinguishing characteristic of the PAF-AH family, compared with other members of the phospholipase A_2 superfamily, is its remarkable specificity for the type of the *sn*-2 residue to be hydrolyzed. Four PAF-AHs have been described in mammals; two of these enzymes belong to the phospholipase A_2 subfamily designated group VII, and the remaining two have been classified as group VIII phospholipases A_2 (3). Group VII enzymes are known by their common names plasma PAF-AH (PLA2G7, also referred to as lipoprotein-associated phospholipase A_2, $LpPLA_2$) and the liver type II PAF-AH (PAFAH2). Sequence analyses of PLA2G7 and PAFAH2 reveal that these genes have a higher degree of homology to neutral lipases and esterases than to other members of the phospholipase A_2 superfamily (4). Indeed, the recent elegant solution of the crystal structure of the plasma PAF-AH to 1.5 Å (5) shows it to have a classic lipase α/beta-hydrolase fold. The type I and II intracellular PAF-AHs constitute the phospholipase A_2 group VIII. These two enzymes are related to each other; they do not share sequence homology with PAF-AHs in group VII; and, in contrast to the group VII enzymes, they are completely specific for PAF (6).

LOCATION, LOCATION, LOCATION

The initial nomenclature chosen to refer to various PAF-AHs did not accurately reflect the actual distribution of these enzymes in tissues and body fluids. For example, the plasma isoform is expressed by macrophages, which retain a portion of the activity, while the type II liver isoform is found in several soft tissues (7), particularly those rich in epithelial cells (8). Comprehensive analyses of the distribution of PAF-AHs have not been conducted, but this can be anticipated by the distribution of their mRNAs. For example, a search of the Gene Expression Omnibus database of high throughput gene expression data (www.ncbi.nlm.nih.gov/geo) reveals robust expression of PLA2G7 mRNA throughout the brain, white adipose tissue, and placenta. Similarly, PAFAH2 mRNA encoding the liver type II enzyme is expressed in liver, kidney, and testis, and less often in brain structures. This approach also reveals that mRNA for group VIII phospholipase A_2 PAFAH1B2, initially purified from the brain, is expressed in diverse human tissues.

The plasma PAF-AH circulates in association with LDL particles and a subfraction of HDL particles that also contain

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apoE (2). Macrophages secrete the largest amount of enzyme and so are the likely source of the circulating enzyme (9). Accordingly, studies in humans receiving allogeneic bone marrow transplants demonstrate that cells of the hematopoietic system, and not hepatocytes, account for all of the plasma enzyme (10). This conclusion was obtained using subjects deficient in plasma PAF-AH activity owing to homozygous point mutations that abolish enzymatic activity (11), whereby the presence or absence of circulating PAF-AH activity correlates with the donors' genotype and was unrelated to that of the recipients (10).

Physical location plays a critical role in the effectiveness of plasma PAF-AH and probably also for the type II enzyme. Two-thirds of the plasma PAF-AH protein associates with LDL and one-third with HDL, but at low PAF concentrations that mimic physiologic levels the enzyme associated with HDL particles is inactive. The inability of HDL-associated PAF-AH to hydrolyze low levels of PAF is not related to the enzyme per se, the same enzyme is bound to both types of particles, but rather is due to the lipoprotein environment that, apparently, limits access of PAF to the enzyme's active site (12). The enzyme rapidly transfers between LDL and HDL particles, and since only about one in a thousand LDL particles carries a molecule of PAF-AH, HDL may function to distribute the enzyme among individual lipoprotein particles. The structural features directing plasma PAF-AH to LDL and HDL particles have recently been reviewed (13), and now can be understood from the solution of the enzyme's structure (5). The intracellular type II enzymatic activity also functions in more than one compartment. This enzyme is distributed between the cytosolic and membrane fractions under basal conditions, but cytoplasmic enzyme migrates to the membrane fraction when cells are subjected to oxidative stress (14). This localizes the enzyme with its substrates, thereby increasing the effectiveness of substrate hydrolysis.

SUBSTRATE SELECTIVITY

Group VII and VIII PAF-AH enzymes are Ca^{2+}-independent phospholipases A_2 that effectively hydrolyze the short sn-2 residue of PAF, yet completely lack activity against membrane phospholipids that bear unmodified, long-chain sn-2 residues. The group VIII enzymes are even more selective as they display complete specificity for hydrolysis of the acetyl residue of PAF (15). Group VII plasma and type II enzymes, however, also hydrolyze unmodified sn-2 fatty acyl residues up to 5 or 6 carbon atoms long, but this length restriction is greatly relaxed when the ω-end of the sn-2 residue contains oxidized (i.e., aldehydic or carboxylic) functions (16). For example, phospholipid oxidation products of the abundant sn-2 linoleoyl-containing phospholipids harbor 9-carbon-long ω-aldehyde or carboxylate functions, and these oxidatively truncated phospholipids are efficiently hydrolyzed by PAF-AH. In fact, the length restriction is fully lost when the sn-2 group contains oxidized functionalities in other positions along the acyl chain, allowing plasma PAF-AH to hydrolyze phospholipids containing C_{18} hydroperoxyoctadecadienoyl (17) and F_2-isoprostane C_{20} (18) residues. Truncated phospholipids with shortened and oxidized sn-2 residues are generated by oxidative attack on phospholipids bearing sn-2 polyunsaturated fatty acyl residues, so the substrate selectivity of the group VII enzymes allows them to function as oxidized phospholipid phospholipases that are specific for oxidatively modified substrates in a sea of unmodified membranous phospholipids. This selective oxidized phospholipid phospholipase function (16) has been demonstrated using human LDL exhaustively oxidized with $CuSO_4$ in the presence and absence of an irreversible inhibitor of plasma PAF-AH. These results (19) show that oxidation results in the loss of a substantial fraction of the diunsaturated and polyunsaturated phosphatidylcholines, with formation of oxidatively truncated phospholipid products. This approach also shows that the LDL-associated plasma PAF-AH is the activity that depletes LDL particles of oxidatively truncated phospholipids, as predicted from prior work with pure standards of these derivatives (16).

IDENTIFIABLE ROLES OF PAF-AHS

Inflammation

PAF is a remarkably potent and common activator of inflammatory cells owing to the generalized expression of the PAF receptor by cells of the innate immune system (1, 2). Accordingly, hydrolysis of this phospholipid autacoid to inactive acetate and a lysolipid by extracellular or intracellular PAF-AHs is predicted to suppress inflammatory signaling. Indeed, expression of plasma PAF-AH is increased by stimulation with LPS and other inflammatory agonists, and decreased by anti-inflammatory drugs and cytokines (13). The first evidence for an anti-inflammatory function was reported in preclinical models of pleurisy and paw edema in which animals pretreated with recombinant plasma PAF-AH displayed marked reductions in PAF-induced vascular leakage (20). The critical role of circulating PAF-AH in controlling PAF receptor ligands was emphasized in an important following study in which the recombinant enzyme was shown not only to protect animals from the consequences of lethal PAF administration, but to also decrease mortality in a model of systemic anaphylactic shock (21). Accordingly, a recent study (22) shows a strong negative correlation between the severity of anaphylaxis and circulating PAF-AH levels. These results bring PAF research full circle in that the original biologic activity ascribed to PAF was as the active principle that caused IgE-induced anaphylaxis model of experimental immune complex disease (23), and the purification, cloning, and administration of the recombinant enzyme that inactivates PAF is now found to reduce mortality in a model of anaphylactic shock.

Additional lines of evidence support a role of PAF-AH in the control of acute inflammation. Administration of recombinant plasma PAF-AH significantly reduces mortality in a murine model of intraperitoneal challenge with the bacterial product lipopolysaccharide, and in a sepsis model of cecal ligation and puncture that allows access of the in-

testinal flora to surrounding tissues and the circulation (24). In agreement with these observations, enteral introduction of the recombinant enzyme reduces the incidence of necrotizing enterocolitis (25) and the extent of inflammation and tissue pathologies in models of acute pancreatitis (26, 27). Finally, intraperitoneal recombinant PAF-AH reduces liver oxidative stress, cell death, and the attendant regeneration of lost tissue following acetaminophen intoxication (28).

Plasma PAF-AH activity modulates chronic inflammatory disease in addition to its efficacy in the acute models of inflammatory insult previously identified. Continual injection of recombinant PAF-AH reduced the incidence of diabetes in diabetic prone BB rats, suggesting that the reduction of either PAF or PAF-like oxidized phospholipids by the circulating recombinant enzyme protected β cells from insulitis (29). Experimental atherosclerosis is more intensively investigated as a model of chronic inflammatory disease because of its relevance to the human condition. Here, too, administration of exogenous plasma PAF-AH reduces inflammatory indices, and delays or reduces progression of vascular damage. Adenoviral infection with a plasma PAF-AH chimera increases circulating PAF-AH activity (30), and reduces monocyte adhesion and homing to atherosclerotic lesions of the vascular wall of apoE-null animals that are prone to atherosclerotic lesion formation (30). An extension of this work (31) shows that PAF-AH adenoviral transfection inhibits injury-induced neointima formation and inhibits spontaneous atherosclerosis in apoE-deficient mice. Importantly, these beneficial changes occurred without decreasing the hypercholesteremia of these animals, showing that inflammatory events, and not the amount of circulating lipids, underlies disease progression. Electroporation of the plasma PAF-AH gene into muscle also increases circulating enzyme levels and reduces the mean thickness of the aortic wall in atherosclerosis-prone apoE-null mice (32).

The reduction in atherogenicity in these models derives from plasma PAF-AH remodeling of lipoprotein particles after oxidation, rather than a direct effect on vascular cells, because lipoproteins isolated from animals transiently over-expressing PAF-AH are less able to induce foam cell formation in an in vitro model (33, 34). The kinetics of lipid oxidation during the development of atherosclerotic lesions provide additional important clues regarding the role of PAF-AH in this syndrome. While oxidized cholesterols accumulate as lesions mature (35), production (and destruction) of oxidized phosphatidylcholines is a continuous process observed in early and advanced lesions (35). PAF-AH hydrolysis of oxidized phospholipids has two beneficial effects. This action releases chemically reactive fragments of esterified fatty acids sequestered by the oxidized particles into the aqueous phase so they are no longer positioned to modify the apolipoprotein constituent that directs lipoprotein function and turnover (36). The second beneficial effect is the destruction of oxidatively truncated phospholipids that activate the PAF receptor (37), those that enter cells to initiate the intrinsic apoptotic cascade (38), those that ligate the scavenger receptor CD36 to promote oxidized LDL uptake and foam cell formation (39), and those that stimulate atherosclerosis in currently undefined ways (40).

The role of PAF-AHs in human physiology, as might be expected, is not as clear as in preclinical model studies. Individuals harboring undetectable levels of plasma PAF-AH activity due to a point mutation near the active site of the enzyme have been described in Japanese (and several other) populations (2). This deficiency, when homozygotic, is associated with small increases in a significant range of cardiovascular and thrombotic diseases (13) but does not lead to rampant inflammation. A countervailing argument is that plasma PAF-AH (also known in this context as lipoprotein-associated phospholipase A_2) contributes to the process of atherogenesis. As previously noted, plasma PAF-AH levels correlate with cardiovascular risk, but whether this enzyme actively contributes to the process or simply has increased in response to the chronic inflammatory environment is unknown. The hypothesis that the enzyme has a direct pathologic role is being tested in human subjects through chronic administration of the inhibitor darapladib. Reduction of the plasma activity in humans by 60% by the drug modestly changed plaque composition as assessed by intravascular ultrasound, albeit without affecting circulating inflammatory markers or plaque volume or deformability (41). Ultimately, the discordant views that plasma PAF-AH is pro- or antiatherogenic will be resolved through further human experimentation with the drug or in heterozygotic individuals that also only possess half the normal activity levels.

Apoptosis

PAF induces apoptosis in a way that is independent of its receptor, and over-expression of the PAF-specific group VIII PAF-AH blocks this (42). Moreover, over-expression of either the plasma (43–45) or liver type II (14, 46) PAF-AH blocks apoptosis in response to diverse physiologic and pharmacologic agents. The clear implication of these data is that the short-chain phospholipid substrates of these enzymes are distinctly proapoptotic and that these lipids play a large part in regulated cell death. Studies using purified truncated phospholipids demonstrate that these molecules are targeted to the mitochondrial compartment, that they depolarize and damage this organelle, and that their presence activates caspases of the intrinsic apoptotic cascade (38). PAF has also been shown to induce apoptosis, but the mechanism underlying this function remains to be elucidated (42). *S. pombe*, which possesses the smallest known eukaryotic genome, still express an isoform of PAF-AH that prevents oxidant-mediated death (47). This suggests that hydrolysis of oxidized phospholipids is a primordial function of PAF-AH. Correspondingly, ablation of the type II PAF-AH sensitizes mice to the toxic effects associated with CCl_4-induced oxidant stress in the liver (8), showing that this protective role is retained by the mammalian enzyme. These combined observations point to an essential role of PAF-AHs as scavengers of apoptotic, short-chained phospholipids.

Control of continual intracellular PAF production

Recent studies have revealed that PAF synthesis takes place in the absence of cellular activation (48). This con-

tinual PAF synthesis and degradation occurs not only in cells of the innate immune system but also in erythrocytes. This process, therefore, seems to be a generalized event that probably reflects phospholipid remodeling whereby a large pool of acetyl-CoA promotes the formation of PAF and its acyl analog (49). Importantly, the amount of PAF accumulated by neutrophils in this way is equivalent to that induced by agonist stimulation. Mammalian cells silence this potential for basal PAF signaling through the enzymatic activity of plasma PAF-AH retained in the intracellular compartment, as demonstrated by immunoblot and inhibitor studies (48).

INCOMPLETELY DEFINED ROLES OF PAF-AHS

The circulating levels of this enzyme correlate with LDL cholesterol levels, so it is sensitive to inflammatory and atherogenic stimuli. Circulating PAF-AH has proven to be a robust, and independent, marker of cardiovascular disease risk (50). Inflammatory regulation of PAF-AH production may account for this correlation, but a countervailing argument has been advanced proposing that PAF-AH contributes to the pathogenesis of atherosclerosis through its production of choline lysolipids. This argument, however, does not consider that the levels of circulating lysolipids already are several orders of magnitude higher than that of PAF, so their hydrolysis would contribute little to the circulating lysolipid pool. Additionally, the biological activities associated with the substrates of PAF-AH are orders of magnitude higher than those elicited by the lysolipid products of the reaction. Other incompletely defined, but important, roles of PAF-AH occur in spermatogenesis and in development, as revealed by the recent genetic targeting of the group VIII enzyme in mice and a type II homolog in *C. elegans*.

EMERGING ROLES OF PAF-AHS

So what does the family of PAF-AHs really do? While many of the functions of these unique phospholipases A_2 are well characterized, a number of important issues remain to be addressed. These include additional insights on the roles of PAF-AHs in apoptosis, and the consequences of continuous PAF production and its potential for intracellular signaling. In addition, future studies must address molecular mechanisms underlying regulation of PAF-AH levels in cardiovascular disease settings. Finally, future studies need to elucidate the impact of location on enzymatic activity and substrate accessibility, along with better characterization of PAF-AH substrates. Studies in humans and in experimental animals still have much to tell us.

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