Platelet-activating factor: a phospholipid autacoid with diverse actions

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Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a potent biological mediator that exerts its effects in a variety of cells and tissues (1–3). This phospholipid mediator was discovered during concurrent investigations of a factor derived from the blood of rabbits undergoing anaphylaxis that activated platelets, and an endogenous polar lipid from kidney that lowered blood pressure. When the common structures were elucidated, a new field of research had emerged (reviewed in 1, 2 by the leaders of the two groups that determined the structure). The trivial name, PAF, is a misnomer as it describes only one of the compound's many effects. This name, however, has been retained due to the lack of a suitable alternative. Other acronyms that include structural information, e.g., AGEPC (alkyl glycerol ether phosphoryl choline) and PAFacether, have been proposed as substitutes but have not found wide acceptance.

PAF is unique in its role as a phospholipid that functions as an intercellular mediator, and it may also function as an intracellular messenger. It acts through a specific receptor, for which a cDNA has been cloned and expressed in mammalian cells. PAF is synthesized by one of two pathways, the first involving the remodeling of cell membrane phospholipids by the hydrolysis of an arachidonate from the *sn*-2 position and its replacement with an acetate. The second route entails its de novo synthesis from 1-O-alkyl-*sn*-glycero-3-phosphate through the incorporation of an acetate, removal of the phosphate, and its replacement with phosphocholine. PAF is degraded by the removal of the acetate and re-acylation with a fatty acid.

The structural elements of PAF are each important for optimal biological activity. Modification of the ether-linkage of the fatty alkyl group at the *sn*-1 position of the glycerol backbone, the short chain acyl residue (acetate) at the *sn*-2 position, or the phosphocholine head group at the *sn*-3 position typically result in compounds with substantially reduced potency (see chapters 7 and 8 in ref. 2). The informal convention is to refer to 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine as PAF, without regard for the length or the degree of unsaturation of the alkyl chain at the *sn*-1 position, and to refer to structurally related compounds as PAF analogs. These PAF analogs may have substantial bioactivity and participate in signaling in vivo. Some of the most recent findings were presented at the Fourth International Congress on PAF and Related Lipid Mediators in September, 1992 and will be published as brief reports in a special issue of the *Journal of Lipid Mediators*.

Metabolism of PAF

Synthesis. PAF can be made by two distinct routes: the remodeling and de novo pathways (**Fig. 1**). The remodeling pathway was the first to be described and is thought to be more important in various inflammatory and allergic responses. PAF is not produced to an appreciable extent via this pathway in unactivated cells. The regulation of its synthesis has been studied intensely for some time and there are recent exciting results. The synthesis is initiated by an arachidonate-specific phospholipase A_2 (PLA_2), as numerous studies have shown that arachidonate release and PAF synthesis are closely coupled. It was proposed that the PLA_2 hydrolyzes arachidonate from 1-O-alkyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine generating 1-O-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (lyso-PAF) and free arachidonic acid (4). The latter can be enzymatically converted to eicosanoids, which also have diverse potent actions. The lyso-PAF can then be acetylated by acetyl coenzyme A:lyso-PAF acetyltransferase to form PAF. This enzyme also is activated by phosphorylation upon cell stimulation. Thus, this pathway yields precursors of two classes of bioactive lipid that are related in many cases by common metabolic pathways and similar effector mechanisms.

Abbreviations: PAF, platelet-activating factor; PLA_2, phospholipase A_2; PKC, protein kinase C; DG, diglyceride; IP_3, inositol triphosphate; CoA-IT, coenzyme A-independent transacylase; LDL and HDL, low and high density lipoproteins, respectively; PE, phosphatidylethanolamine.

Remodeling

De Novo
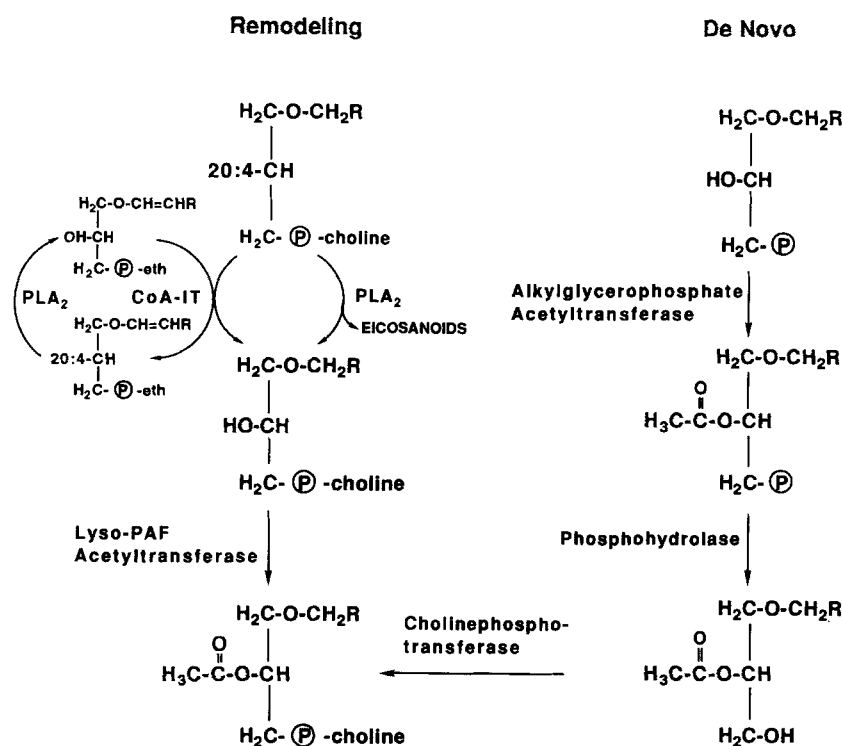
Fig. 1. Pathways for synthesis of PAF. Remodeling pathway (on left): This is the pathway by which most PAF is thought to be made, particularly in conditions of inflammation and allergic response. PAF synthesis is initiated by the action of phospholipase A$_2$. Shown on the right hand side of this pathway is the traditional view that the PLA$_2$ acts on 1-O-alkyl-2-arachidonoyl glycerophosphocholine to yield lyso-PAF and free arachidonate. The arachidonate then is converted to eicosanoids. The lyso-PAF is acetylated by a specific acetyltransferase using acetyl coenzyme A as a donor. A newly recognized mechanism, shown as a cycle on the left, utilizes a PLA$_2$ that acts on arachidonate-containing plasmalogen phosphatidylethanolamine to release free arachidonate. The lyso-phosphatidylethanolamine then serves as an acceptor for arachidonate in a transacylation reaction from the PAF precursor, which yields lyso-PAF. This mechanism fits a large body of data on arachidonate trafficking. In any case, this PLA$_2$ step (or the two steps) is absolutely required for initiation of PAF synthesis; we have termed it *conditional.* It requires activation of the synthesizing cell with a rise in free intracellular calcium and activation of protein kinases that increase the activity of PLA$_2$. Once the lyso-PAF is generated, the acetyltransferase reaction becomes limiting. The activity of this enzyme also is regulated by cell activation, probably by a phosphorylation/dephosphorylation cycle. We have termed this a *modulating* step. De novo pathway (right side): In this pathway 1-O-alkyl-sn-glycero-3-phosphate is acetylated by 1-O-alkyl-sn-glycero-3-phosphate:acetyl coenzyme A acetyltransferase. 1-O-Alkyl-2-acetyl-sn-glycero-3-phosphate phosphohydrolase then produces 1-O-alkyl-2-acetyl-sn-glycerol which can be converted to PAF by a dithiothreitol-insensitive CDP-cholinephosphotransferase. The enzymes in this pathway seem to act constitutively and be regulated only by the availability of substrate. It has been proposed that this pathway may continuously produce a small amount of PAF to serve a physiological role(s).

The PLA$_2$ that initiates PAF synthesis is the subject of considerable research effort currently. Whole-cell experiments have demonstrated that the PLA$_2$ is activated through a receptor-linked G protein acting through a protein kinase. Studies in endothelial cells showed that this G protein is not inhibited by pertussis or cholera toxins (5). Alternatively, the receptor may be bypassed by directly activating with ionophore. By either pathway the synthesis of PAF requires Ca^{2+} influx. In recent years several mammalian PLA$_2$s have been purified, including high molecular weight proteins from macrophages (6, 7), U937 monoblasts (8, 9), kidney (10), and platelets (11). This class of enzymes is the best candidate to date to be respon-sible for PAF synthesis. These enzymes display characteristics that are consistent with in vitro data compiled on the release of arachidonic acid and production of PAF. They require physiological levels of Ca^{2+} to translocate to the membrane (7, 12), where the substrate is located, although the Ca^{2+} may not be directly involved in the catalysis. They are selective for arachidonate-containing phospholipids, but do not show much selectivity for the fatty chain linkage in the sn-1 position, and hydrolyze phosphatidylethanolamine and phosphatidylcholine equally well. The 110 kDa enzyme purified from U937 cells has now been cloned (13, 14) and the cDNA expressed in CHO cells (15). Experiments performed with

this system demonstrated that cells expressing the high molecular weight protein, but not those transfected with the low molecular weight (secretory) PLA$_2$ cDNA or untransfected cells, respond to ATP or thrombin stimulation with release of arachidonate. In whole cells the activation of PLA$_2$ depends on PKC, which may directly phosphorylate PLA$_2$ or, alternatively, another kinase that then acts on PLA$_2$.

High levels of a smaller, secreted PLA$_2$ have been detected at sites of inflammation and it has been purified from several sources (16–18). Although this enzyme clearly catalyzes a reaction that yields the lysophospholipid intermediate for PAF synthesis, the evidence strongly suggests that the larger cytoplasmic PLA$_2$ is the relevant one for PAF synthesis, at least under regulated conditions.

It now appears that the initial step in PAF synthesis may not be as simple as the hydrolysis of arachidonate from a common precursor. Recent studies have shown that a coenzyme A-independent transacylase (CoA-IT) also is involved in the production of PAF. Studies in neutrophil homogenates were unsuccessful at showing a PLA$_2$ activity in vitro with the expected characteristics. Further, others had shown that the ethanolamine plasmalogens were major sources of released arachidonate in stimulated neutrophils (19). In support of this observation, whole cell experiments showed a remarkable build-up of 1-*O*-alk-1'-enyl-2-lyso-*sn*-glycero-3-phosphoethanolamine but not of lyso-PAF (20). Venable et al. (21) found that adding excess lyso-PAF to PLA$_2$ assays to trap the radiolabeled lyso-PAF product led to a striking increase in hydrolysis of 1-*O*-alkyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine. This hydrolysis, however, was not catalyzed by a PLA$_2$ but by the CoA-IT. These observations led to the proposed model of PAF synthesis shown in Fig. 1 (22). In this model arachidonate-containing plasmalogen phosphatidylethanolamine is hydrolyzed by a PLA$_2$. The lyso-PE product stimulates the transfer of arachidonate from the PAF precursor to the lyso-PE, forming lyso-PAF in the reaction. This reaction is catalyzed by the CoA-IT. Similar results were obtained independently in several laboratories (23–26). A PLA$_2$ is still required for the initiation of PAF synthesis but it may not act directly on 1-*O*-alkyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine, the PAF precursor. Importantly, the transacylation reaction itself does not result in the production of free arachidonic acid and therefore does not fit the definition of a phospholipase. The CoA-IT is apparently not up-regulated upon Ca^{2+} stimulation of neutrophils (M. E. Venable and R. L. Wykle, unpublished results) but catalysis is increased by lysophospholipids generated by a PLA$_2$. A PLA$_2$ with specificity for choline plasmalogens was reported by Hazen, Stuppy, and Gross (27), and a similar activity for ethanolamine plasmalogens was described by Okazaki et al. (28). An enzyme with this specificity would both initiate the pathway using the transacylase and, if the

latter reaction were saturated, would yield a lysoplasmalogen substrate. Other data indicate that this step may be regulatory in PAF synthesis, at least under some conditions (24). It is still to be seen whether this pathway exists in other cell types and how large a role it plays in the production of PAF in light of the recent findings on PLA$_2$. This alternate pathway raises the possibility of another target for the inhibition of PAF synthesis, in addition to lyso-PAF acetyltransferase, without affecting arachidonate release.

In the next step of PAF synthesis lyso-PAF is converted to PAF by the transesterification of acetate, a reaction catalyzed by a specific acetyl-coenzyme A: lyso-PAF acetyltransferase (29) which is specific for short chain acyl-CoAs. The enzyme also has a preference for choline-containing glycerophospholipids and, in particular, the lyso-PAF (30). This selectivity is only modest, however, and does not appear to account for the specific synthesis of PAF compared to PAF analogs.
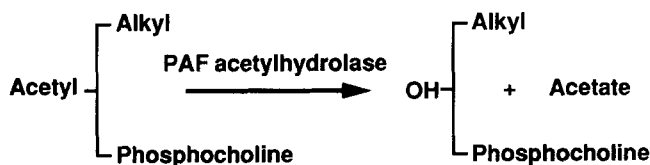
This lyso-PAF acetyltransferase also is activated by phosphorylation. Whole-cell experiments have implicated the involvement of protein kinase C in the regulation of the acetyltransferase, although the effect observed may have been primarily at the PLA$_2$ step. Cell-free phosphorylation experiments suggested the involvement of protein kinase C, protein kinase A, and/or calmodulin-dependent protein kinase (31–33). From these various data we have proposed that activation of the PLA$_2$ step is a conditional step, i.e., it is absolutely required for the synthesis of PAF, while activation of the lyso-PAF acetyltransferase modulates the amount of PAF produced from the lyso-PAF precursor (30). This modulation is essential because synthesis of an excessive amount of PAF could lead to pathologic conditions, while too little could impair normal homeostasis.

PAF can also be synthesized by a de novo pathway (Fig. 1). In this pathway 1-*O*-alkyl-*sn*-glycero-3-phosphate is acetylated by 1-*O*-alkyl-*sn*-glycero-3-phosphate:acetyl coenzyme A acetyltransferase. 1-*O*-Alkyl-2-acetyl-*sn*-glycero-3-phosphate phosphohydrolase then produces 1-*O*-alkyl-2-acetyl-*sn*-glycerol which can be converted to PAF by a dithiothreitol-insensitive CDP-cholinephosphotransferase. The enzymes in this pathway are apparently constitutively active and seem to be regulated largely by the availability of substrate (34). It has been suggested that the de novo pathway continuously produces a small amount of PAF to serve some physiological role. However, a recent study reported that the activity of a key enzyme, the acetyltransferase, which is a distinct enzyme from the transferase in the remodeling pathway, was increased in activated endothelial cells (35). Studies showing the synthesis of PAF from 1-*O*-alkyl-*sn*-glycero-3-phosphate under various conditions will help our understanding of the role of the de novo pathway in homeostasis.

Degradation. PAF made by either pathway is degraded to inactive product by one of a family of phospholipases, the

PAF acetylhydrolases (**Fig. 2**). This activity, which is found in a variety of cells and tissues (36–38), does not require Ca^{2+} and catalyzes the hydrolysis of short sn-2-acyl groups only (36, 39). The enzymes hydrolyze the acyl analog of PAF as well as phospholipids containing oxidatively fragmented sn-2-fatty acyl groups (40–42). The acetylhydrolase also hydrolyzes the acetate of ethanolamine-containing analogs of PAF. The activities in plasma and in cells have identical substrate specificity, but studies of the molecular weight, chemical inhibition, protease inactivation, and antibody recognition have shown the enzymes to be distinct (36, 38). The plasma protein is resistant to treatment with sulfhydryl and histidyl reagents, proteolysis, and NaF. In contrast, the activities in spleen, liver, and leukocytes are inhibited or at least partially sensitive to all these except NaF. The red cell enzyme shows still different characteristics in that it is inhibited by histidine and cysteine modification, and is sensitive to proteolysis and NaF. While the neutrophil and erythrocyte enzymes migrated at the same rate in native-PAGE electrophoresis, the liver enzyme displayed a higher mass/charge ratio (38).

The plasma PAF acetylhydrolase has a molecular mass of 43 kDa and is tightly associated with LDL and HDL (39, 43). Under optimal catalytic conditions the HDL- and LDL-associated enzymes exhibit identical catalytic properties. However, when concentrations of PAF described in vivo (10^{-9} M) are added to plasma, the HDL-associated enzyme does not degrade PAF at appreciable rates while the LDL-associated enzyme does (44). It is unclear what accounts for the different activity in the two types of particles under conditions of limiting substrate, but we suspect that it has to do with partitioning of the PAF. The cellular source(s) of plasma PAF acetylhydrolase probably is macrophages (45, 46) and hepatocytes

(47, 48), both of which synthesize and secrete an activity with properties identical to the plasma enzyme. In both cases, the secretion of the enzyme is independent of lipoprotein particles, but the acetylhydrolase preferentially associates with either nascent lipoproteins secreted by the cells (in the absence of serum in the medium) or with lipoproteins in the medium. Macrophages also may play a role in the local regulation of PAF levels since the precursor monocytes do not produce the enzyme. However, upon differentiation to macrophages they begin to produce and secrete PAF acetylhydrolase (45, 46). Secretion of the PAF acetylhydrolase by hepatocytes is suppressed by estrogen (48), which may account for the dramatic fall in the plasma activity that has been observed late in gestation, and which has been proposed to be important in the initiation of labor (49). Administration of glucocorticoids increased the level of the PAF acetylhydrolase in the plasma of rabbits and reversed the suppressive actions of estrogen (50). This suggests that a portion of the anti-inflammatory actions of these compounds may be due to an increase in this enzyme that catalyzes the removal of inflammatory lipids.

After PAF is inactivated by the acetylhydrolase the resultant lyso-PAF is reacylated by one of three routes: a CoA-dependent acyltransferase, a CoA-dependent transacylase which does not require Mg^{2+} or ATP, as does the acyltransferase, or by a CoA-independent transacylase (51, 52). In resting cells the last pathway predominates; however, after stimulation all three pathways apparently participate (4, 53).

PAF analogs

It has been known for several years that PAF is part of a family of structurally related compounds (54, 55). The importance of these related compounds is now being re-emphasized. First, it is now recognized that related compounds are made in more significant amounts than was earlier appreciated. Second, structure/function studies traditionally have focused primarily on the ability of PAF and its analogs to cause aggregation or secretion of platelets or neutrophils. Recent findings have suggested other possible activities or roles for these analogs. Synthetic structural analogs have been useful for the study of the PAF receptor and enzymes that metabolize PAF, as well as showing promise for potential therapeutic agents.

The acyl analog of PAF (1-O-acyl-2-acetyl-sn-glycero-3-phosphocholine, acyl-PAF) is the major acetylated lipid produced by stimulated mast cells, basophils, and endothelial cells. Triggiani et al. (56) suggested that these cells form part of a class of cells distinct from inflammatory cells such as neutrophils, eosinophils, and macrophages that selectively produce PAF. The preference for making PAF versus acyl-PAF apparently reflects the relative abundance of the respective phospholipid precursors (56–59). Studies on the cytosolic

Fig. 2. Degradation of PAF. PAF is degraded by hydrolysis of the acetyl residue at the sn-2 position. This reaction is catalyzed by a specific family of phospholipases referred to as PAF acetylhydrolases. The form found in plasma is tightly associated with low density and high density lipoproteins, but is synthesized and secreted independently of the apolipoproteins. It does not require calcium for activity and is highly selective for very short acyl groups, in particular, phospholipids with long chain fatty acids at the sn-2 position are not utilized as substrates. We recently have shown that it also catalyzes the hydrolysis of phospholipids with short remnants of oxidatively fragmented fatty acids at the sn-2 position, which may be important in protecting against oxidative damage of the LDL particle. Related activities with the same substrate requirements have been described in a variety of cells and tissues, but their other properties demonstrate that they are different proteins from the plasma enzyme.

PLA$_2$ demonstrated enzymatic specificity for arachidonate but limited selectivity for *sn*-3 group and no specificity for the linkage in the *sn*-1 position. The enzyme that catalyzes the next step, lyso-PAF acetyltransferase, has only a modest preference for choline over ethanolamine and for lyso-PAF compared to the acyl compound (30). Consistent with these in vitro results, stimulated neutrophils have been shown to produce a significant amount of 1-*O*-alk-1'-enyl-2-acetyl-*sn*-glycero-3-phosphoethanolamine (55, 60). The function, if any, of this compound is unknown. The potential function of the acyl analogs has been studied in more detail, although not extensively. One set of studies on the role of acyl-PAF implicated it as a priming agent in neutrophils (61), while others demonstrated that pre-treatment with acyl-PAF antagonized some actions of PAF (62). However, based on the experimental protocol, this result could have been due to desensitization of the receptor by pre-treatment with acyl-PAF. Finally, others have suggested that it could participate in neutrophil adhesion to endothelial cells (57, 63, 64). Triggiani, D'Souza, and Chilton (65) have shown a distinct catabolic pathway for acyl-PAF since unlike PAF it is a substrate for phospholipase A$_1$.

The membrane phospholipids that can serve as precursors to PAF and acyl-PAF predominantly contain polyunsaturated fatty acids in the *sn*-2 position. These fatty acids are susceptible to free radical oxidation during pathologic conditions in which a strongly oxidative environment occurs: reperfusion injury of ischemic tissue, the adult respiratory distress syndrome, and chronic inflammation. We found that exposure of pure synthetic phospholipids to oxidative stress strong enough to fragment the unsaturated acyl chain results in compounds that are structurally similar to PAF, and act through its receptor to exhibit the biological activities of PAF (66). Tokumura et al. (67) had identified similar compounds with dramatic physiological actions in extracts of brain. We recently found that similar compounds are generated when cultured endothelial cells are exposed to oxidants, and that they are secreted into the medium as vesicles that are "blebbed" from the surface (68). This class of compounds is made by a nonenzymatic free radical reaction and therefore could be generated in much larger amounts than is PAF (or analogs). They likely are less potent than PAF but, if produced in large enough quantities, could have important pathological actions. Further, the production would not be subject to the regulatory controls that govern PAF synthesis, and the PAF acetylhydrolase might therefore be essential in limiting their actions by catalyzing their removal.

PAF geography

PAF was discovered as a soluble mediator and has been found in a variety of body fluids, so it is apparent that some cells secrete it after synthesis. However, we found that endothelial cells secrete little or none of the PAF they synthesize (69), which led to a reassessment of this issue. It now is clear the percentage secreted varies dramatically in different cells and under different conditions (69–74). Bratton et al. (72) found that a change in membrane symmetry, such as has been observed during cell activation, can influence PAF secretion as opposed to retention. Miwa et al. (73) recently discovered a specific protein, in addition to albumin, that binds PAF and appears to facilitate its secretion. The findings that much of the PAF synthesized in many cells remains associated with the cells and indirect evidence for an intracellular PAF receptor (discussed below) indicate that PAF may serve as an intracellular messenger in some systems (75, 76). Testing this possibility will depend on the development of potent, selective ways to inhibit PAF synthesis, but this remains an interesting and potentially important possibility.

Another mechanism by which PAF exerts its actions after synthesis in endothelial cells, and perhaps others, results from its location on the surface of the cells where it serves as an intercellular messenger. In activated endothelial cells a glycoprotein, P-selectin, is rapidly expressed on the surface where it tethers passing leukocytes, and simultaneously the PAF that is synthesized is transferred to the surface and activates the neutrophils (77–79). A similar mechanism has been described for the adhesion of platelets to neutrophils, PAF on the surface of the latter activates the platelets (80, 81). We assume that the PAF must transfer from the synthesizing cell to the receptor on the target cell without being in the "fluid" phase, but this has not been demonstrated directly. Obviously this would require a restricted environment between the two cells.

The intracellular location of PAF synthesis has not been unequivocally determined. However, the high molecular weight PLA$_2$, which probably initiates PAF synthesis, is cytosolic in resting cells and translocates to an unidentified intracellular membrane in response to calcium. The precursor to PAF, alkyl-acyl glycerophosphocholine, has been reported to be in intracellular compartments in neutrophils (82); however, there can be substantial lipid redistribution during subcellular fractionation (M. E. Venable and R. L. Wykle, unpublished results). The other enzymes of PAF synthesis are located in the endoplasmic reticulum and perhaps also in other intracellular organelles (83, 84). In metabolic labeling experiments PAF appears first in the phagolysosomal fraction or, alternatively, in the endoplasmic reticulum depending on the stimulus and is then transferred to the plasma membrane (85). Thus, PAF must be transported from its site of synthesis to the plasma membrane for secretion or for expression on the surface. This could occur by at least two mechanisms, each of which may operate under different conditions. PAF could be carried between membranes by a specific transport protein, as

happens with other phospholipids. Once there it would flip to the outer leaflet. This scenario is supported by the existence of a transport protein that is selective for PAF and is induced during differentiation (86, 87). Alternatively, the movement of PAF from an intracellular organelle to the plasma membrane could occur by fusion of lipid vesicles. This possibility is supported by the observation that PAF made by neutrophils may be found in vesicles similar to those known to be involved in membrane cycling (88). This is an attractive possibility since it is the mechanism for secretion and/or surface expression of many other compounds.

PAF receptor

PAF acts via specific receptors on the membranes of responsive cells (see ref. 89 for review). This was one of the early surprises in this field as PAF is a phospholipid and can simply partition into the membrane. Nonspecific surface binding, in fact, led to much difficulty in elucidating the structure of the receptor. However, specific receptors have been described in the many cells and tissues in which PAF has effects. Attempts to purify the receptor were unsuccessful because the detergent micelles in which it was solubilized readily incorporated PAF nonspecifically during the binding assay. Further, most responsive cells have only several hundred to a few thousand receptors, so the starting material was quite limited.

Fortunately, the receptor recently was cloned independently by two approaches, expression cloning and G protein-linked receptor sequence homology, yielding the same gene product (90–93). In the original report of work done in guinea pig lung, Northern blots showed hybridization with 2.2, 3.0, and 4.0 kb mRNAs (90). Subsequent work on human tissues have shown hybridization with a single message of about 4 kb (92, 93). There is a single copy present in the genome. The receptor message was detected in guinea pig leukocytes, spleen, lung, and kidney but not in intestine, liver, heart, or brain, whereas among human tissues the mRNA was only observed in placenta and lung but not in heart, brain, liver, skeletal muscle, kidney, or pancreas. This finding is surprising because some of these tissues that did not have PAF receptor message are known to respond to PAF. This discrepancy may have been due to problems with detection at low copy numbers or may support the findings of Hwang (94) which suggest a second, possibly internal, PAF receptor.

The human receptor gene encodes a 342 amino acid protein of 39 kDa with greater than 80% identity with the guinea pig protein. Computer analysis of the protein sequence revealed limited sequence homology with other G protein-linked receptors. The highest degree of identity, 29%, was with the N-formyl peptide receptor. The protein was predicted to have seven membrane-spanning domains as has been shown for other G protein-linked receptors.

It was also shown to have a conserved aspartic acid in the second membrane-spanning domain, two cysteines in the second and third extracellular loops, which form a disulfide bond, and three prolines in the sixth and seventh transmembrane domains, which may play a role in ligand binding. The aspartic acid in the third lipid domain, which is believed to participate in ligand binding in related receptors, is missing. Another highly conserved amino acid in this family is the asparagine in the seventh helix, but in the PAF receptor an aspartic acid is found in this position. Interestingly, this change has been found in only one other receptor, that for thromboxane A_2, another lipid mediator. The potential N-terminal, N-glycosylation site on the guinea pig protein was not seen on the human sequence. There were several potential serine and threonine phosphorylation sites, which may be involved in down-regulation. The cDNA was also functionally expressed in *Xenopus laevis* oocytes (90, 91) and in mammalian cell lines (COS-7 fibroblast-like cells [91, 93] and HL-60 cells [92, 95]). The receptor displayed specific and saturable PAF binding that was competed by PAF receptor antagonists and down-regulated by repeated treatment. Binding studies in wild-type cells using radiolabeled receptor antagonists, many of which displayed less nonspecific binding, reinforced the results (96).

Many antagonists of the PAF receptor have been described (see 2, 97 for reviews) including structural analogs and several classes of compounds without any structural relationship. The latter often were discovered by screening natural products and traditional therapies for asthma and inflammatory disorders. Potent, selective antagonists were crucial in early studies of the receptor, and remain valuable in studies of the pathophysiology of PAF. Some have shown promise as therapeutic agents. Endogenous inhibitors have been described in tissues and their structures, precise mechanism of action, and physiological role are subjects of continuing study.

PAF binding to transfected cells elicited voltage and Ca^{2+} responses (92, 95). GDPBS was also shown to repress the electrical response to PAF administration, further supporting the coupling of the PAF receptor to a G protein (90, 91). Stimulation of transfected cells with PAF induced inositol trisphosphate (IP_3) synthesis (91) supporting previous work which showed that the binding of PAF activates phosphatidylinositol-specific phospholipase C (chapter 9 of ref. 2), leading to the transient production of (IP_3) and diacylglycerol (DG). In vitro work on cells that normally express the PAF receptor also demonstrated that IP_3 mediates the release of internal calcium stores, which is followed by an influx of extracellular calcium. The rises in DG and calcium activate protein kinase C (PKC), which catalyzes phosphorylation of intracellular proteins. Activation of PKC by other routes also down-regulates the PAF receptor (98, 99), implying that the

response to PAF may be mediated by PKC. If so, this would indicate a complex role for PKC: it transmit signals in response to PAF and then shuts off subsequent responses. After down-regulation, receptors reappear on the cell surface by a process that requires synthesis of new receptors (100). PAF also stimulates arachidonate hydrolysis from phospholipids, initiating eicosanoid synthesis thereby accounting for some of the actions of PAF (101, 102). In human neutrophils exogenous PAF induces additional synthesis of PAF (103, 104), which requires the activation of a phospholipase A_2 (PLA_2). Whether this additional PAF can stimulate the cell of its origin is unclear, however, in light of the data showing receptor down-regulation. In addition to the well-characterized signal transduction through G proteins and PKC, recent studies have shown that PAF also activates a tyrosine protein kinase (105, 106), which may be an essential component of some responses including induction of c-fos and c-jun.

Scatchard analysis of the binding data from cells transfected with the PAF receptor showed a single class of binding sites with a K_d near 1 nM as was seen in previous studies of the native PAF receptor (90, 92, 93). The K_d correlates well with the responses by whole cells to various levels of PAF. Kunz, Gerard, and Gerard (93) conducted binding experiments at 4°C and at 22°C and found an 8- to 10-fold difference in the number of binding sites, suggesting a second type of PAF receptor. Hwang's studies (94) revealing differences in the rank order of potency for several receptor antagonists between neutrophils and platelets also suggested that there may be at least two types of receptors. One of these may be expressed internally. Indeed, studies have begun to explore this role for PAF (107).

Pathological and physiological actions

PAF has many actions in addition to activation of platelets. It functions in normal physiological processes such as inflammation, hemostasis, and several aspects of reproduction. However, its role in the mediation of pathological responses including asthma, ischemia, gastric and pulmonary distress, allergy, and shock (108 and chap. 10 in ref. 2) have made it the focus of intense research. The role of PAF in normal cell function and in disease has been recently reviewed in detail (89, 109) and will only be covered briefly here. In vivo PAF causes increased vascular permeability, hypotension, decreased cardiac output, stimulation of uterine contraction, gastrointestinal disorders, acute bronchoconstriction, and leukocyte adhesion to endothelial cells. PAF has been found in patients undergoing sepsis, and PAF receptor antagonists impart significant protection in animal models of sepsis (110). Also, many symptoms of sepsis can be mimicked by administration of PAF (110). In vitro PAF can cause activation of platelets, polymorphonuclear leukocytes, mono-

cytes, and macrophages and stimulation of glycogenolysis in perfused liver.

These effects are achieved at threshold concentrations ranging from 10^{-12} to 10^{-9} M. PAF has been shown to be produced during a number of the pathological and normal physiological processes described above. Several approaches have been used to study the role of PAF, for example, its administration mimics some pathological conditions. In other experiments, pharmacological agents that antagonize the binding of PAF to its receptor have been found to attenuate or reverse certain pathological processes where PAF is the suspected mediator (109). Finally, correlations between the process under study and the level of PAF in tissues or a relevant fluid may reveal connections. This has been difficult for PAF because it has been hard to measure (this is made worse due to its actions at such low concentrations), and because it is readily metabolized. The concentration of PAF in cells or tissues represents the net difference between synthesis and degradation. Changes in the levels or activation state of the relevant enzymes have been associated with certain conditions. In particular, changes in the activity of the PAF degrading enzyme, plasma PAF acetylhydrolase, have been associated with certain diseases and conditions (111–115). Moreover, PAF or PAF-like lipids were found to be elevated in the plasma of smokers (114). Miyaura, Eguchi, and Johnston (116) found a substance in cigarette smoke extract that inhibits the PAF acetylhydrolase. Thus, if this unidentified compound were to inhibit the degradative enzyme at the time when PAF, or a PAF-like lipid, was being generated, there could be markedly increased levels of PAF leading to vascular damage. Recent experiments in our laboratory have shown that PAF acetylhydrolase protects LDL from free radical oxidation by hydrolyzing oxidatively fragmented fatty acyl residues from the sn-2 position of phospholipids (117). These oxidized lipids are also good substrates for the PAF acetylhydrolase (42). This action may help prevent LDL from being recognized by the macrophage scavenger receptor, which is an early step in atherosclerosis. PAF may also be important in inflammation of the lung since its administration induces asthma-like conditions and edema (118). Monocytes, which generate PAF, are seen in inflamed lung; however, after differentiating to macrophages, they begin to secrete PAF acetylhydrolase suggesting that the differentiation process may serve an anti-inflammatory role, at least with respect to these lipids.

PAF is usually viewed as a mediator of pathological events, at least in part because of the circumstances of its discovery. However, there are substantial data supporting a role for it in physiological events. In such a view, the pathological actions would result from excessive production, or production under inappropriate conditions, or lack of degradation; some escape from normal regulatory

mechanisms. One system that has been studied in detail is reproduction. O'Neill (119) has provided evidence that fertilization of eggs and implantation and growth of the embryo are stimulated by PAF. Likewise, PAF may be involved at the end of pregnancy as it is a potent stimulus for uterine contraction. During the late stages of pregnancy PAF is generated by fetal lung but does not stimulate uterine contractions because it is hydrolyzed by high levels of maternal PAF acetylhydrolase (49). Near the end of gestation the enzyme is down-regulated by exposure to estrogen (48–50, 111), which allows the PAF synthesized by the fetus to accumulate and to stimulate uterine contraction.

Future challenges

The actions of PAF are intertwined with many other cell-signaling mechanisms. This and its diverse effects have made dissection of its pathophysiological role(s) difficult, as was the case for many years for eicosanoids, another group of lipid mediators. The new tools that are now available such as genetic probes, recombinant proteins, and specific pharmacological agents should speed the work. Some of the most important questions that will be addressed in the near future include: *1)* How is PAF receptor expression and function regulated, and to which G protein is it linked? *2)* What is the mechanism of PAF secretion and cell-surface expression? *3)* What role does intracellular PAF have in signaling? *4)* How are the enzymes involved in PAF metabolism regulated, and can they be manipulated pharmacologically? *5)* Do PAF analogs contribute significantly to PAF signaling or do they have distinct roles in biology? ▮▮

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