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PLATELET-ACTIVATING FACTOR AND RELATED LIPID MEDIATORS

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■ **Abstract** Platelet-activating factor (PAF) is a phospholipid with potent, diverse physiological actions, particularly as a mediator of inflammation. The synthesis, transport, and degradation of PAF are tightly regulated, and the biochemical basis for many of these processes has been elucidated in recent years. Many of the actions of PAF can be mimicked by structurally related phospholipids that are derived from nonenzymatic oxidation, because such compounds can bind to the PAF receptor. This process circumvents much of the biochemical control and presumably is regulated primarily by the rate of degradation, which is catalyzed by PAF acetylhydrolase. The isolation of cDNA clones encoding most of the key proteins involved in regulating PAF has allowed substantial recent progress and will facilitate studies to determine the structural basis for substrate specificity and the precise role of PAF in physiological events.

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INTRODUCTION

Platelet-activating factor (PAF) is the trivial name for a phospholipid, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine that has potent, diverse physiological actions. Its discovery and the early studies to characterize its actions, synthesis, and degradation were described in a previous chapter in this series (1) and other reviews (2–5). Many lipid mediators are derived from phospholipids (6), but PAF was the first one shown to have autocoid, or messenger, functions as an intact phospholipid. This signaling results from PAF binding to a specific receptor—not from physicochemical effects on the plasma membrane of the target cell. One of the most intriguing aspects of PAF has been the strict structural requirement for binding to its receptor and for recognition as a substrate both by synthetic and degradative enzymes. The last point seems to be an eminently practical requirement because, if the degradative enzymes were not specific for this unusual structure, there would be continuous hydrolysis of structural phospholipids.

PHYSIOLOGIC ACTIONS OF PLATELET-ACTIVATING FACTOR

PAF's primary role seems clearly to be to mediate intercellular interactions; when synthesized by cells of a variety of types, it binds to receptors on the plasma membranes of other cells, which activates them and changes their phenotypes. In addition to intercellular signaling, it has been proposed that PAF has both autocrine and intracrine effects, the latter occurring via one or more intracellular receptors. Although postulated to exist for many years based on pharmacological studies, intracellular receptors for PAF are yet to be rigorously characterized. We focus here on intercellular signaling by PAF. Many of its best-characterized signaling roles are in the vascular and inflammatory systems (see below), but it also transmits information between cells in the central nervous system and in endocrine, gastrointestinal, and other organs (see review articles above). Most of the following examples are drawn from studies in inflammation, but the principles likely are relevant in multiple systems. Several mechanisms regulate the PAF intercellular-signaling system. These include tightly controlled synthetic pathways, spatial regulation of the display and biologic availability of PAF, cell-specific expression of the receptor for PAF, homologous and heterologous desensitization of the receptor, and rapid degradation of PAF by extracellular and intracellular acetylhydrolases. Each of these regulatory features is considered in more detail elsewhere in the chapter. Together, they indicate specialization of function for PAF as an intercellular signal, and the redundant mechanisms appear to have evolved to precisely control its biologic activities. Thus, unregulated or dysregulated signaling by PAF can be a mechanism of disease. Disease models in isolated cell systems (7–10) and experimental animals (5) and observations in human syndromes (5) also support this possibility.

PAF Is a Cell-Associated Signal for Leukocyte Adhesion and Activation in Models of Acute Inflammation: Juxtacrine Signaling

In the inflammatory response, PAF has well-characterized actions, mediating cell-cell interactions in models of acute and chronic inflammation in virtually all organs. The cells involved include endothelial cells, leukocytes of several classes, and others (5). These studies have shown that PAF can mediate paracrine signaling by acting over short distances in solution. In some cases, PAF may also circulate and act in an endocrine fashion. The latter mode of action is controlled by plasma PAF acetylhydrolase, which limits the half-life of PAF to a few minutes in the blood of humans and experimental animals (see below). Experiments in models of acute inflammation have also shown, however, that most signaling by PAF may occur between closely juxtaposed cells and that it can be recognized by its receptor on target cells while associated with the plasma membrane of the signaling cell. Thus, PAF can signal in a juxtacrine fashion (Figure 1). These models have also demonstrated that PAF acts cooperatively with an adhesion protein, P-selectin, on the endothelial cell surface and that activation responses triggered by signals delivered via the PAF receptor in the target cell further modify the intercellular interaction.

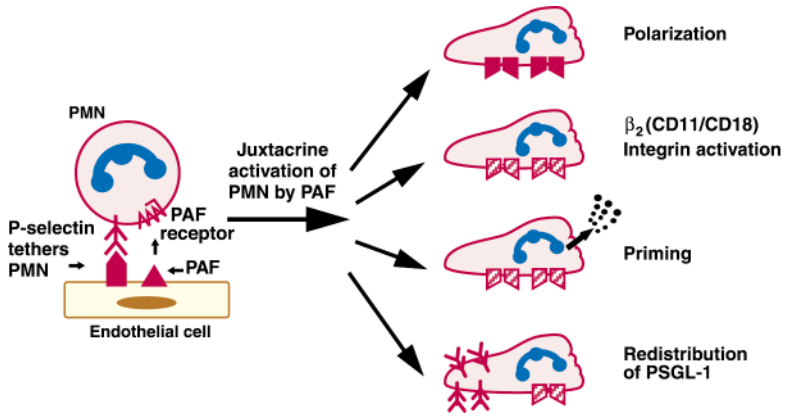

Figure 1 Platelet-activating factor (PAF) signals the priming and activation of leukocytes at the surfaces of inflamed human endothelial cells (a juxtacrine system for spatial control of inflammation). PAF and an adhesion protein, P-selectin, are coordinately displayed on the plasma membranes of stimulated human endothelial cells. P-selectin tethers the leukocyte to the endothelial cell, which allows the PAF from the endothelial cell to bind to its receptor on the polymorphonuclear leukocyte. This constitutes a form of juxtacrine signaling, which may be a general way to spatially restrict the actions of a potent, pleiotropic mediator such as PAF. β_2 integrins are not shown on the polymorphonuclear leukocyte (*left side* of the figure), for convenience. This figure is reproduced from previously published work (10a), with permission of the publisher.

PAF had been shown in early studies to be a fluid-phase mediator that could activate platelets and various leukocytes in suspension (1). Subsequently, it was demonstrated that PAF is synthesized in regulated fashion by endothelial cells stimulated with thrombin or other inflammatory mediators and that this PAF activated polymorphonuclear leukocytes (PMNs) (11–13), which are key effector cells in the acute inflammatory response. This was the first observation of synthesis of a signaling factor for PMNs by inflamed endothelial cells, and it established a molecular mechanism for local activation of leukocytes at the endothelial surface rather than requiring the diffusion of chemotactic factors into the blood. This local mechanism of leukocyte signaling had been predicted by classic in vivo studies but never proven. The adhesion of PMNs in response to PAF is mediated by activation-dependent alterations in the affinity and avidity of β_2 integrins on their surface (14). PAF also induces other responses of PMNs that are critical in acute inflammation, including cell polarization, enhanced motility, priming for enhanced granular enzyme release, and redistribution of surface ligands (Figure 1). In addition, PAF activates other leukocytes involved in inflammation, such as monocytes, where PAF signals NF-κB translocation to the nucleus and alterations in gene expression (15) and other functional responses.

A key additional observation was that PAF synthesized by stimulated endothelial cells is not released into solution but that almost all of it remains cell-associated even in the presence of albumin, an acceptor molecule to which it binds avidly (13, 16, 17). Furthermore, a significant fraction of PAF that is synthesized by stimulated endothelial cells is translocated to the outer surface of the plasma membrane, where it is available for binding to its receptor on target PMNs (18). The specific mechanism of translocation to the endothelial surface and whether PAF is localized in hydrophobic patches or is noncovalently linked to a presenting protein are currently unknown. A variety of experimental strategies were used to demonstrate that PAF that is endogenously synthesized by endothelial cells and is retained at their surfaces ligates the PAF receptor on target PMNs. These strategies included receptor blockade, receptor desensitization, and degradation of PAF in the endothelial plasma membrane with extracellular preparations of PAF acetylhydrolase (18). These experiments demonstrated that PAF displayed by inflamed endothelial cells has the requisite features of a juxtacrine signaling molecule (19). Subsequent experiments have confirmed that PAF can operate in this fashion when displayed by stimulated endothelial cells both in static systems and when the target PMNs are subjected to flow to model in vivo conditions (20–23; reviewed in 24). Recently, strategies with exogenous recombinant PAF acetylhydrolase (25) or PAF receptor antagonists (26) demonstrated that PAF is a juxtacrine signaling molecule at the surfaces of activated human platelets, which—like endothelial cells—are critical in cell-cell interactions in inflammatory and thrombotic responses (27, 28). These findings suggest that this mode of action, presentation of PAF on the cell surface by the cell originating the signal with transfer to an adjacent cell that has a receptor, may be a general mechanism. If so, it has profound implications for how this mediator and other small signaling molecules function in many tissues. In essence,

it would make the concentration, as determined in biological fluids such as blood, irrelevant because the crucial determinant would be its relative abundance in a pseudo-two-dimensional space. This could have attractive implications for signaling events such as occur in the neuronal synapse and other environments where spatial limitation of the signal would be important.

Signaling Roles for PAF in Vivo

Many studies involving exogenous administration of PAF or its endogenous generation in experimental animals indicate that it has signaling roles in vivo (reviewed in 5). Again, many of these involve models of inflammation or inflammatory injury. Recent studies with genetically manipulated mice (29, 30) further document signaling roles of PAF in vivo. In the first, the guinea pig PAF receptor was overexpressed in mice by Shimizu and colleagues (29), who originally cloned the guinea pig receptor (31). The transgenic animals had increased mortality when challenged with endotoxin, developed melanocytic tumors of the skin, and had increased bronchial hyperreactivity in response to inhaled methacholine. Increased bronchial reactivity and susceptibility to endotoxin were consistent with previous studies in wild-type animals, where there is considerable evidence that dysregulated signaling by PAF or PAF-like lipids occurs in endotoxemia and models of asthma (5). Melanocytic tumors were, however, unexpected although there is evidence that PAF has neoplastic effects, and it is known to mediate skin inflammation. In a converse strategy, the same group created mice with targeted deletions in the PAF receptor (30). These PAF receptor knockout animals were developmentally normal and reproduced effectively, indicating that some of the actions in which PAF had been implicated previously are not essential actions. Future studies with these mice are important because more sensitive assays might detect subtle alterations in reproductive capacity, neurological function, and other normal physiological events in which PAF might play a role. In the reported studies, the knockout animals were tested in a model of inflammation and had much milder anaphylactic responses to exogenous antigen challenge than did wild-type animals, including less cardiovascular instability, airway constriction, and alveolar edema. These results were again consistent with findings in earlier animal models, involving receptor blockade or other manipulations of the PAF system, and were also consistent with the respiratory physiology of the transgenic mice overexpressing the PAF receptor. In contrast, the knockout animals remained susceptible to endotoxin, with vascular and cytokine responses equivalent to those in the paired animals with intact PAF receptors. This suggested that PAF is not required for endotoxic shock, although it is a modulating signal (30). A potentially important variable is that the genetic background of mice used in this study was different from that of the transgenic mice that overexpressed to PAF receptor.

In a third study, Shimizu and colleagues examined inflammatory acute lung injury caused by acid aspiration in knockout mice and in transgenic mice overexpressing the PAF receptor. The injury was reduced in animals deficient in the PAF

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