Platelet-activating factor in inflammation and pulmonary disorders

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INTRODUCTION
Since its recognition as a substance released from rabbit basophils after IgE stimulation that is capable of causing platelet aggregation [1, 2], platelet-activating factor (PAF, 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) has continued to generate interest as a potent mediator of inflammation. Compared with other lipid mediators of inflammation, such as those generated from the cyclo-oxygenase or lipoxygenase pathways, PAF has a wider range of inflammatory effects on many cell types, such as activation of neutrophils, eosinophils, lymphocytes and macrophages, increased adherence of various inflammatory cells to the vascular endothelium, inducing microvascular leakage, and 'priming' of inflammatory cells. Within a few years of its discovery, PAF was implicated as a mediator of inflammation in various diseases with a chronic inflammatory component. With respect to diseases of the lungs, asthma has figures prominently, but PAF has also been implicated in pulmonary hypertension and the adult respiratory distress syndrome (ARDS). The recent availability of specific, potent PAF antagonists has given further impetus to the investigation of the role of PAF in these conditions [3, 4]. Coupled with this opportunity for the investigation of the role of PAF in disease, there are increasing new data on the biological actions of PAF, including a potential intracellular role and its interactions with cytokines which indicate the complexity of any potential role for PAF in health and disease. Several reviews have already been published on the subject of PAF [5–9]; in this review, I will bring together some of the more recent observations, including the potential effects of PAF antagonists in pulmonary disease.

METABOLISM OF PAF
Biosynthesis
PAF is synthesized by either remodelling or de novo pathways (Fig. 1) [10]. A two-step process is involved in the remodelling pathway with the activation of a phospholipase A2 to hydrolyse a long-chain acyl group from 1-alkyl-2-acyl-sn-glycero-3-phosphocholine in membrane phospholipids to form 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (lyso-PAF), followed by acetylation by an acetyltransferase enzyme. Acetyltransferase activity can be induced by the calcium ionophore A23187, tumour necrosis factor and interleukin-1α, partly by an increase in synthesis of the enzyme [11, 12]. This activity has been demonstrated in many inflammatory cell types in vitro, including neutrophils, eosinophils, platelets, macrophages and endothelial cells. Activation of the remodelling pathway results in the biosynthesis of excessive amounts of PAF.

By contrast, the de novo pathway involves the direct generation of PAF from ether-linked phospholipids, such as alkylacylglycerols, under the action of a highly specific cholinephosphotransferase present in several tissues, including rat lung and human neutrophils [13, 14]. This enzyme is not affected by inflammatory stimuli and is thought to be important for the production of PAF at basal physiological levels.

Catabolism
PAF is rapidly inactivated by the removal of the acetate moiety to form lyso-PAF, which is biologically inactive, by an acetylhydrolase that has been described both intracellularly and in plasma [15,
Plasma acetylhydrolase activity is found in lipoprotein fractions [17]. Cellular acetylhydrolase activity may be released into the supernatant after stimulation of platelets with various agonists, including PAF [18]. Both plasma and cellular acetylhydrolase activities may therefore control PAF levels in the plasma and intracellular compartments. Bronchiolar and alveolar epithelial cells rapidly take up PAF administered by the intratracheal route, with metabolism to lyso-PAF and phosphatidylcholine [19]. Inflammatory cells, such as neutrophils, also rapidly incorporate PAF into its fattyacyl derivative [20]. Lyso-PAF can be acylated to 1-alkyl-2-acyl-sn-glycero-3-phosphocholine with membrane or microsomal preparations and can be converted into alkylglycerols by the combined actions of lysophospholipase D and phosphohydrolase [21].

The important regulatory controls in the metabolism of PAF have not been completely elucidated. Many of the enzymes involved in the remodelling route of PAF biosynthesis require the presence of Ca\(^{2+}\), whereas those in the de novo pathway are inhibited by Ca\(^{2+}\). Phosphorylation of the acetyltransferase enzyme in the remodelling pathway to the catalytically active unit appears to require a cyclic AMP-dependent kinase [22]. It is also possible that protein kinase C activity is required for coupling a rise in intracellular Ca\(^{2+}\) concentration to the initiation of PAF and leukotriene (LT) \(B_4\) biosynthesis [23]. In endothelial cells, the initiation of PAF production is regulated by guanine nucleotide-binding proteins (G-proteins) which mediate entry of extracellular Ca\(^{2+}\), with a subsequent elevation of intracellular Ca\(^{2+}\) concentration [24].

Although acetylhydrolase is important in controlling PAF levels in both plasma and intracellular compartments, other enzymes, such as lysophospholipase D, may be important in regulating the PAF precursor pool of ether-linked phospholipids in membranes [25]. Of clinical interest is the finding that serum PAF acetylhydrolase activity is reduced in asthmatic children [26], but the clinical significance of this finding remains unclear.

**Other ‘platelet-activating factors’**

Although most studies have concentrated on two molecular species of PAF (i.e. \(C_{16}\) and \(C_{18}\) PAF), stimulated inflammatory cells can also synthesize and release a variety of PAF homologues and analogues, including saturated and unsaturated alkyl-chain homologues and 1-0-acyl-PAF analogues and acetylated glycercyolphosphoethanolamine [27–29]. Some human cell types, such as neutrophils, eosinophils and macrophages, produce almost exclusively PAF on stimulation, whereas other cell types (mast cells, basophils and endothelial cells) produce predominantly 1-acyl-2-acetyl-sn-glycero-3-phosphocholine [30]. This profile of PAF generation also depends on the stimulus; for example, human basophils synthesize predominantly 1-acyl-2-acetyl-sn-glycero-3-phosphocholine in response to anti-IgE and predominantly PAF in response to the calcium ionophore A23187. Lung tissue can also be stimulated via cell-surface IgE to produce a large proportion of 1-acyl-2-acetyl-sn-glycero-3-phosphocholine relative to PAF [30].

The biological activities of these PAF analogues and homologues remain poorly studied. Alkyl-chain PAF homologues are also active in stimulating...
human neutrophils in vitro when compared with C_{16} PAF. On the other hand, 1-acyl-2-acetyl-sn-glycero-3-phosphocholine is a relatively weak stimulus for the human neutrophil and platelet, and may inhibit some of the stimulating effects of PAF on the human neutrophil. The biological and pathophysiological roles played by these 'platelet-activating factors' require further elucidation.

**Cellular sources**

A wide range of cell types produce PAF in vitro in response to several stimuli. Within a few minutes of activation of neutrophils by opsonized zymosan or the calcium ionophore A23187, PAF is synthesized, although only 3–4% is released [31]. A non-metabolizable bioactive analogue of PAF can stimulate human neutrophils to synthesize PAF, as detectable by incorporation of [^{3}H]acetate into PAF [32]. In addition, LTB_{4} also stimulated PAF biosynthesis and 5-hydroxyicosatetraenoic acid synergistically increased PAF-stimulated PAF synthesis [32], and PAF itself has been shown to stimulate PAF acetyltransferase in neutrophils [33]. These lipid mediators may therefore be linked in a network to enhance the inflammatory response in vivo, with PAF being capable of enhancing its own generation. The cytokine granulocyte–macrophage colony-stimulating factor (GM–CSF) can augment the production of PAF and increase the activity of acetyltransferase in human neutrophils [34]; this effect may depend on protein synthesis and phospholipase A₂ activation [35].

Human eosinophils obtained from normal subjects exhibit no detectable acetyltransferase activity, but eosinophils from patients with eosinophilia show high activity and release PAF after stimulation with various chemotactic factors [36]. Of all circulating cells, human eosinophils contain the most abundant PAF precursor, 1-acyl-2-acetyl-sn-glycero-3-phosphocholine [37]. Purified human hypodense eosinophils release PAF after IgE- (but not IgG-) mediated activation [38].

Human alveolar macrophages obtained by bronchoalveolar lavage of allergic asthmatic patients also release small amounts of PAF after stimulation in vitro but not with zymosan [39]. Human alveolar macrophages also produce and release a large amount of lyso-PAF after stimulation with the calcium ionophore A23187 or after IgE-mediated mechanisms [39]. It has been suggested that lyso-PAF could be used by other cells, such as neutrophils, which can take up lyso-PAF. Such a transcellular biosynthesis of PAF has been described between platelets and neutrophils [40]. Cultured human endothelial cells release a small percentage of synthesized PAF after activation by a calcium ionophore, bradykinin, thrombin and interleukin-1 [41, 42]. Purified human lung mast cells also generate PAF when stimulated with anti-IgE, but most of the PAF is also retained intracellularly [43]. A human lung epithelial cell line has been shown to generate PAF [44]. Human lymphocytes appear to lack acetyltransferase activity [45]. However, under specific circumstances, such as Fc receptor stimulation, PAF can be released by large granular lymphocytes [46].

PAF biosynthesis can be stimulated in human monocytes in response to the cytokines interleukin-1β, tumour necrosis factor and interferon-α, in a biphasic process. During the first 2 h of stimulation most of the PAF is retained, whereas during the late phase of activation (6–8 h), PAF was largely secreted [47]. New protein synthesis was required for the late peak of PAF synthesis [47].

**Role for intracellular PAF?**

The recent recognition that many cell types such as neutrophils, endothelial cells and macrophages can produce large amounts of PAF but retain it intracellularly has led to the hypothesis that PAF may have an intracellular role [48]. It has been suggested, for example, that the ability of endotoxin to prime neutrophils such that they respond to stimuli such as formylmethyl-leucylphenylalanine with an enhanced secretion of superoxide anions may be related to the primed increase in intracellular PAF by endotoxin in response to formylmethyl-leucylphenylalanine [49]. Therefore, intracellular PAF may modulate the secretory events of the neutrophil. It has been suggested that PAF may act as an intracellular second messenger [50]. Thus, in bovine endothelial cells and guinea-pig macrophages, stimulation with the calcium ionophore A23187 and bradykinin induced PAF synthesis, followed by prostacyclin (PGI₂) generation, effects which were inhibited by PAF receptor antagonists. Exogenous PAF did not activate PGI₂ metabolism in these cells, suggesting that PAF may be acting on intracellular receptors [50].

There is also evidence to suggest that intracellular PAF in endothelial cells stimulated by agents such as 'trombin or leukotrienes may mediate neutrophil adherence, but more direct proof is needed [51, 52]. One possible advantage for PAF to remain cell-associated is that it may be protected from degradation by the neutrophil.

**CELLULAR ACTIVATION**

**PAF receptors**

The stereoselectivity of the effects of PAF, its high biological potency and the development of specific tachyphylaxis suggest that the surface membrane receptors are involved in mediating these effects. With the use of [^{3}H]PAF as a radioligand, high-affinity binding sites on human and rabbit platelets
PAF receptor subtypes

The existence of PAF receptor subtypes was first proposed on the basis of the different potency of the PAF antagonist kadsurenone in inhibiting PAF-induced chemiluminescence of guinea-pig peritoneal macrophages and aggregation of pig peripheral leukocytes [58]. On the basis of relative potencies of several PAF antagonists in inhibiting binding and functional assays and of the differential sensitivities to pertussis and cholera toxins or G-protein activation, it was suggested that the PAF receptor and its G-protein coupling on human polymorphonuclear leukocytes are structurally different from those on human platelets [59]. It has also been suggested that PAF receptor subtypes may mediate different functional responses of the eosinophil, such as superoxide anion generation and release of eosinophil peroxidase [60]. However, different intracellular activation pathways may also underlie these findings. Whether the different molecular species of PAF act through the same PAF receptor is not known.

The cloning and expression of a complementary DNA for a PAF receptor from guinea-pig lung may provide much stronger evidence for the existence of subtypes [61]. The receptor was found to be a member of the super-family of receptors coupled to GTP-binding proteins. The hydropathy profile of the PAF receptor, with seven putative membrane-spanning α-helices, is typical of other G-protein-linked receptors.

Intracellular mechanisms

The inhibition of binding of PAF to its specific receptors by GTP indicates that the PAF receptor is coupled to a G-protein [55]. In addition, PAF stimulates GTPase activity in cell membranes from human platelets and neutrophils [59, 62]. However, the nature of the G-protein is not certain and PAF receptors may be linked to different intracellular pathways in different cell types. PAF increases the intracellular level of free Ca^2+ and stimulates the turnover of polyphosphoinositides in a variety of cells [63–67]. In guinea-pig eosinophils, PAF-induced receptor-mediated formation of inositol triphosphate mass and subsequent release of intracellular calcium stores, which was linked to degradation of the eosinophil [68]. Mobilization of Ca^2+ has also been shown when B-lymphocytes are stimulated with PAF, an effect accompanied by transcription of the c-fos proto-oncogene, which is involved in the control of the proliferative response [69].

Effects on cells

PAF has potent effects on a wide variety of cells. At picomolar concentrations, PAF causes aggregation of washed rabbit and human platelets, with the release of 5-hydroxytryptamine (serotonin) [70]. The stimulation of neutrophils by PAF results in the release of lysosomal enzymes and superoxide anions, the generation of LTB_4 and chemotaxis [71–73]. PAF also increases the production of superoxide anion by human alveolar macrophages in a dose-dependent manner [74]. PAF is extremely potent in causing the release of the granule-associated enzyme eosinophil peroxidase from human and guinea-pig eosinophils [75]. Activation of eosinophils by PAF induces tracheal epithelial shedding in vitro, associated with a slowing of ciliary beat frequency, an effect inhibited by PAF antagonists [76]; a lesser degree of damage was observed with neutrophils activated by PAF. In addition, it induces a dose-dependent enhancement of eosinophil cytotoxicity, as measured by the killing of Schistosoma mansoni schistosomula coated with C3b, IgG and IgE antibodies [77]. PAF can also up-regulate IgE binding of normodense human eosinophils [78]. PAF is a potent chemotactic and chemokinetic mediator for eosinophils in vitro [73] and promotes the adhesion of neutrophils and eosinophils to vascular endothelial cells, primarily through an effect on the endothelial cell [79, 80]. In addition, nanomolar concentrations of PAF cause a retraction of endothelial cells in culture with rearrangement of the cytoskeleton and induces an increase in albumin transfer across endothelial cell monolayers [81], observations which may underlie the increase in vascular permeability in vivo.

PAF may also regulate cellular immune responses [82]. PAF inhibits human lymphocyte proliferation when stimulated with mitogens such as phytohaemagglutinin or concanavalin A and also suppresses interleukin-2 production [83]. At higher concentrations of PAF, CD4^+ T-cell proliferation is inhibited [83]. PAF induces suppressor cells, accompanied by an increase in CD8^+ T-cells and a small decrease in CD4^+ T-cells [84]. PAF can also inhibit the intracellular metabolism of PAF in neutrophils [53] and human neutrophils [54] have been demonstrated. This binding appears to be specific and can be inhibited by PAF antagonists. However, there is generally a high level of non-specific binding. In addition, PAF is rapidly metabolized. For example, in the neutrophil, [3H]PAF is rapidly converted into its acyl derivative at 37°C and transferred into specific granules, but this metabolism is not present at 4°C [54]. Labelled PAF antagonists have proved to be more suitable as radioligands. For example, [3H]dihydrokadsurenone binds specifically to rabbit platelet membranes with saturation and is displaced by PAF and other PAF antagonists [55]. Specific binding to isolated neutrophils and eosinophils of [3H]WEB 2086, which has a relatively low non-specific binding related to its low lipophilicity, has been demonstrated [56, 57].
enhance the production of immunoglobulins of both IgG- and IgE-secretory B-cell lines [85].

Interaction of PAF with cytokines and arachidonic acid products

PAF is probably best considered as a mediator acting among a network of mediators involved in chronic inflammatory processes. This is best illustrated by the interactions of PAF with a range of cytokines and with arachidonic acid products. Cleavage of the preformed precursor of PAF liberates two important precursor molecules, lyso-PAF and arachidonic acid, which are then metabolized to PAF and lipooxygenase and/or cyclo-oxygenase products. For example, LTB₄ and PAF production from neutrophils stimulated by the ionophore A23187 are tightly coupled in time course [86]. PAF itself can stimulate LTB₄ synthesis in the neutrophil and the eosinophil [72, 87]. Similarly, many stimuli for PAF synthesis in the endothelial cell also produce PGH₂ simultaneously [50].

While some cytokines such as interleukin-1β, tumour necrosis factor, interferon-α can induce the synthesis of PAF from human monocytes [47] and GM-CSF can augment the production of PAF from human neutrophils [34], PAF can also increase the release of cytokines such as tumour necrosis factor and interleukin-1 from alveolar macrophages and monocytes, respectively [88–90]. The enhancement of tumour necrosis factor release by PAF may be mediated through the production of LTB₄ [88]. Interactions between PAF and LTB₄ in human neutrophils can be increased by GM-CSF. Preincubation of blood neutrophils with GM-CSF enhances the ability of PAF to stimulate leukotriene synthesis by increasing both arachidonic acid availability and 5-lipoxygenase activity [91]. Basophils primed with interleukin-3, GM-CSF or interleukin-5 can be induced to release histamine and LTC₄ through IgE-dependent mechanisms by PAF, which has no effect on its own [92]. GM-CSF also enhanced PAF-induced eosinophil accumulation in the lungs of guinea-pigs [93]. These observations suggest that a potential mechanism for priming and for enhanced responses during inflammatory response involves the interaction between certain cytokines produced by macrophages or lymphocytes and PAF.

ACTIONS OF PAF IN THE LUNGS

Airways narrowing

PAF induces variable isometric contraction of human bronchial smooth muscle in vitro, with rapid development of tachyphyaxis [94], but other investigators have found that PAF is only active in the presence of platelets [95–97]. Potentiation of the contractile effects of histamine by PAF in human bronchial muscle has also been reported [94]. In vivo, PAF aerosol is a potent bronchoconstrictor in normal subjects, approximately 100-fold more potent than metacholine, with the rapid development of tachyphyaxis [98, 99]. This bronchoconstrictor response is not inhibited by anti-histamines [100] or by thromboxane receptor antagonists [101]; however, antagonists of LTD₄ significantly reduce this response [102, 103], in agreement with the observation that PAF causes an increase in urinary LTE₄ in normal subjects [104, 105]. A PAF antagonist, UK 75,506, inhibited the PAF-induced bronchoconstriction and the increase in urinary LTE₄ excretion [104]. Part of the bronchoconstrictor response to inhaled PAF is therefore mediated through the release of sulphidopeptide leukotrienes, the source of which remains unclear.

Airways microvascular leakage

PAF also induces an immediate increase in microvascular leakage throughout the respiratory tract in the guinea-pig [106–108], an effect independent of circulating platelets and of eicosanoid generation [106]. A delayed increase in airway microvascular leakage of radiolabelled albumin at 5 h has also been observed, associated with concomitant exudation of plasma proteins across the airway epithelium [109]. Whether inhaled PAF can induce similar changes is not known. One could also postulate that such an action in the airways could contribute to airways mucosal thickening and airways narrowing.

Mucus

PAF stimulates the secretion of mucus from explants of trachea of a variety of species including human [110–112], and this appears to be secondary to lipooxygenase pathway activation [111]. In a guinea-pig tracheal tube preparation, PAF increases the protein content of tracheal fluid through an induction of plasma extravasation rather than by mucus secretion [112]. PAF also induces small changes in ion transport and epithelial conductance [112]. Inhaled PAF impairs tracheobronchial mucociliary clearance in normal subjects [113].

Bronchial responsiveness

An increase in bronchial responsiveness to metacholine in normal subjects, sometimes persisting for many days [98], has been described and confirmed by other studies [99, 112, 114]. Others have reported negative findings [101, 115]. Asthmatic patients do not appear to demonstrate any increase in responsiveness after PAF [99, 116]. PAF has
been reported to induce bronchial hyper-responsiveness in a variety of animal species, including guinea-pig, dog and sheep [117-119].

Circulating and bronchovascular cells

PAF causes a profound neutropenia within 5 min of inhalation by normal and asthmatic subjects, with a rebound neutrophilia at 15 min [100, 116]. Circulating neutrophils become hypodense within 15 min of inhalation of PAF as a result of an increase in cell volume [120]. The falls in circulating blood cell levels could be due to a combination of haemodynamic factors, such as slowing of pulmonary blood flow and increased adheriveness of cells to the pulmonary vascular endothelium. Radio-labeled neutrophils transiently accumulate within the pulmonary vasculature after inhalation of PAF [121]. Four hours after inhalation, a significant increase in neutrophil recovery is observed in the bronchoalveolar lavage fluid of normal subjects [120]. It is possible that LTB4 released by PAF may be involved as increased serum levels of LTB4 have been measured after PAF inhalation in normal subjects [112]. Using a skin window technique, selective accumulation of eosinophils has been demonstrated when PAF was injected intradermally in allergic subjects [122]. PAF induces eosinophilia infiltration of the airways of guinea-pigs [123], and whether it produces a similar effect in atopic subjects is not known.

Pulmonary vasculature and microvascular permeability

Infusion of PAF in sheep induces an acute transient rise in pulmonary arterial pressure, an effect abolished or attenuated by cyclo-oxygenase inhibitors [124, 125]. An increase in the lung lymph flow and the lymph-to-plasma protein concentration ratio occurs, indicating an increase in pulmonary macrovascular permeability [124]. A fall in the protein reflection coefficient, a measure of pulmonary vascular permeability, has also been reported in conscious sheep infused with PAF [126]. In guinea-pig and rabbit isolated lung preparations, PAF infusion caused pulmonary oedema, which may have been secondary to the concomitant induction of pulmonary hypertension [127, 128]. In the rat lung, pulmonary oedema induced by PAF is dependent on the generation of leukotrienes [129]. In the same species, extremely low doses of PAF (0.001-1.0 μg per animal) reduced the increases in pulmonary artery pressure induced by hypoxia, prostaglandin F2α or noradrenaline [130]. In the rat, these vasodilator effects are dependent on the presence of an intact endothelium and are independent of cyclooxygenase products [130]. Chronic infusion of PAF into rabbits over a 4-week period resulted in pulmonary hypertension, an enlargement of the right ventricle and internal thickening and a decreased number of small pulmonary arteries [131]. Inhalation of PAF in normal subjects did not induce any significant changes in systemic or pulmonary haemodynamics, but an increase in alveolar–arterial O2 partial pressure gradient and a fall in the arterial O2 partial pressure was found, together with ventilation/perfusion mismatching [132].

PAF IN PULMONARY DISEASES

Because of its wide range of effects on cellular function and on the priming of the inflammatory response, PAF has been considered as a mediator of inflammation. Which of these effects are important in disease processes is unknown, and therefore the role of PAF in vivo is not clear. It is also pertinent to ask whether PAF is a mediator of particular pathological processes, perhaps those involved in particular pulmonary conditions such as asthma, or whether PAF participates generally in chronic inflammatory processes, in concert with other mediators, to provide both beneficial and detrimental effects of inflammation.

Together with its potent effects in the airways and its potential to mimic many of the features of asthma, PAF has naturally been proposed as a mediator in asthma and allergy [133, 134]. Another area of interest is its participation in ARDS, as PAF appears to play a role in animal models of endotoxic shock and as endotoxin may be involved in the pathogenesis of ARDS [135, 136]. Given its potent effect on the pulmonary circulation, PAF has also been implicated in the pathophysiology of pulmonary hypertension.

PAF antagonists

There have been recent improvements in the assays of PAF with the development of radio-immunoassays [137, 138], but the rapid breakdown of PAF in biological fluids by acetylhydrolase and its rapid uptake by surrounding inflammatory cells tend to limit the usefulness of these assays in disease. In some studies, high levels of lyso-PAF have been measured in fluids obtained from the nose after allergen challenge [139, 140] and interpretation of these results is difficult, as lyso-PAF is both a precursor and a metabolite of PAF. If PAF has an important intracellular role and acts mostly within the local milieu of the inflammatory response, measurement of PAF in biological fluids may not provide important information.

The development of PAF antagonists that are capable of blocking the receptor-mediated response of PAF has made it possible to investigate the role of PAF in various animal models of pulmonary
disease and ultimately in human diseases such as asthma. Several classes of antagonists have been described and reviewed [3, 4]. The triazobenzodiazepines, such as WEB 2086, and UK 75,506, developed from a pteridine ring have been tested in humans and found to block PAF-induced bronchoconstriction and neutropenia [141]. These antagonists are currently undergoing clinical trials in patients with asthma.

An alternative approach to the study of the contribution of PAF in disease is to inhibit the biosynthesis of excessive amounts of PAF by blocking an enzymic step in the formation of PAF. Acetyltransferase would be an obvious target for inhibiting PAF biosynthesis via the remodelling route. Such an inhibitor of PAF biosynthesis would be very effective in controlling pathophysiological levels of PAF without affecting the synthesis de novo of PAF required for physiological functions. More selective control of the effects of PAF could be acquired in this way than with the PAF-receptor antagonists. Indeed, if there are several molecular species of PAF which may be active, and if the effects of PAF are mostly intracellular or localized, PAF-receptor antagonists may be less effective by virtue of their selectivity and of the need to achieve very high local concentrations. However, no specific inhibitors of PAF synthesis have been developed for whole-animal or organ studies.

**Asthma and allergic diseases**

The pathogenesis of asthma remains unclear, but recent studies have revealed that there is a complex and distinctive inflammatory process in the airway which is associated not only with the contraction of airway smooth muscle, but also with airway oedema and plasma extravasation, mucus hypersecretion and bronchial hyper-responsiveness. Studies of airway mucosal biopsies from patients with mild asthma have revealed the presence of a chronic inflammatory process characterized by mucosal oedema, infiltration with eosinophils and lymphocytes and epithelial shedding [142–144]. Because PAF may mimic many of these inflammatory features of asthma, it has been implicated in this disease. PAF may interact with eosinophils to cause the release of eosinophil basic proteins which can damage airway epithelium forming the basis for bronchial hyper-responsiveness [8].

Measurement of PAF in biological fluids, such as plasma and bronchoalveolar lavage fluid, remains difficult. Recovery of PAF in plasma and bronchoalveolar lavage fluid has been reported in asthmatic patients as measured by a platelet-aggregating assay, but without confirmation by mass spectrometry [145, 146]. An increase in PAF activity derived from acetylation of lyso-PAF and detected by a platelet-aggregating assay has been observed in the plasma of asthmatic patients during the late asthmatic response, but not in those with a single immediate response at 6h after antigen challenge [45]. In one study, plasma PAF levels increased immediately after allergen challenge of mild seasonal asthmatic subjects, but not after methacholine challenge [146a]. High levels of lyso-PAF have been detected in bronchoalveolar lavage fluid from allergic subjects after allergen challenge, but no significant increase in PAF levels were seen [147]. A sensitive radioimmunoassay technique for PAF has been developed [137].

The effects of PAF antagonists have been studied in various animal models which mimic some of the features of asthma and these experiments have yielded encouraging results. Thus, an inhibitory effect of PAF antagonists has been demonstrated on eosinophil infiltration in the airways and bronchial hyper-responsiveness induced by ovalbumin in sensitized guinea-pigs [123, 148]. In addition, the late phase induced by allergen challenge is also inhibited in allergic sheep and rabbits. However, similar studies in allergic asthmatic patients have not shown significant effects on the late-phase response with the PAF-antagonists WEB 2086 and MK-287, although there is some doubt as to whether adequate blockade of the PAF receptor was achieved [149, 150]. A small inhibitory effect of the PAF antagonist BN 52021 on the acute bronchoconstrictor response to allergen has been reported in asthmatic children [151]. These studies indicate that the PAF antagonists may not be beneficial in asthma. Clinical studies with the PAF antagonists in asthma are currently underway.

**ARDS**

ARDS is a complex clinical syndrome characterized by refractory hypoxaemia and high permeability pulmonary oedema in the presence of a normal pulmonary wedge pressure [152]. This syndrome may result from a complex interaction between mediators such as PAF, eicosanoids, complement factors and cytokines, as well as from their relationships with the effector cells of the inflammation cascade.

Exogenously administered PAF is capable of causing many of the hallmarks of lung injury seen in ARDS, such as alveolar-capillary damage, high-permeability pulmonary oedema, pulmonary vasoconstriction and bronchoconstriction. Lungs taken from PAF-treated animals reveal frank damage of endothelial cells in addition to vascular permeability [153]. Because of the potential contribution of endotoxinaemia to ARDS [154], it is of interest that PAF antagonists have been shown to inhibit several features of lung injury induced by endotoxin in experimental animals [155–158]. For example, a PAF antagonist inhibited hypoxaemia, pulmonary oedema and the increased permeability of the alveolar-capillary membrane after endotoxin-
induced lung injury in pigs [158]. In this study, the level of LTB₄ in the bronchoalveolar lavage fluid was increased and it has been postulated that PAF may mediate lung injury through the release of 5-lipoxygenase metabolites, such as LTB₄ [157, 158]. Increased levels of PAF have been found in blood and lungs of rats during endotoxaemia [155]. PAF may also prime other inducers of pulmonary endothelial injury, such as proteamine sulphate [159], predominantly by enhancing pulmonary venoconstriction.

**Pulmonary vascular disease**

The role of PAF in the regulation of the normal pulmonary circulation remains unclear [160]. Lung homogenates obtained from normal rats contain measurable levels of PAF [156] and PAF is released into bronchoalveolar lavage fluids in rats exposed to hypoxia [161]. High plasma levels of PAF have been detected in persistent pulmonary hypertension in the newborn [162]. However, there is conflicting evidence as to the potential contribution of PAF to hypotoxic pulmonary vasoconstriction. In some experiments, exogenous PAF reversed hypoxic pulmonary vasoconstriction, suggesting a down-regulating role for endogenously released PAF during hypoxia [163, 164]. In isolated perfused rat lung, hypoxic vasoconstriction is reduced by the PAF antagonist WEB 2086 at doses which have little effect on the direct vasoconstrictor effect of angiotensin II [165]. On the other hand, potentiation of the vasoconstrictor response to angiotensin II and alveolar hypoxia in a similar preparation with two other PAF antagonists has been reported [166]. These diametrically opposite observations are difficult to explain.

**CONCLUSION**

The mass of information about PAF that has accumulated highlights the complexity of the biosynthesis, metabolism, cellular response and control of this lipid mediator. Intuitively, one may assume that PAF participates in the inflammatory response as a primitive response of tissues to injury. It is also possible that PAF may have a physiological role in maintaining homeostasis. Of greater interest is the potential contribution of PAF to disease processes, such as in pulmonary disorders. Here the fundamental question is whether PAF participates as a major mediator, being responsible for the initiation of a series of mediator-driven processes of crucial pathophysiological significance in particular diseases, or whether PAF is just one of many mediators in a 'soup' which contribute in concert with many other mediators. While many of the properties of PAF that can be demonstrated in vitro point to some unique features of PAF, the initial studies with specific PAF antagonists in pulmonary conditions, such as asthma, do not indicate that PAF can be singled out as playing a major role. However, it is perhaps too early to reach such a conclusion when many of the complex mechanisms by which PAF activates cells remain to be elucidated.

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Platelet-activating factor


