Does airway smooth muscle care about platelet-activating factor?

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In 1966, BARBARO and ZVAIFLER [1] demonstrated the release of histamine from rabbit platelets during an acute allergic response. Five years later, it was shown that a soluble factor from leucocytes, capable of inducing platelet activation, was responsible for this effect [2, 3]. In subsequent years, the factor was recognized for its potent ability to stimulate platelet aggregation and secretion and was consequently named "platelet-activating factor" (PAF) [4]. By the end of the decade, three independent groups had elucidated its chemical structure as 1-alkyl-2(R)-acetyl-glycerol-3-phosphocholine. On the basis of its structural and physiological properties, the lipid was subsequently termed as PAF-acether ("ace" for acetate and "ether" for the alkyl bond) [5], acetylglyceryletherphosphorylcholine (AGEPC) [6], or antihypertensive polar renomedullary lipid (APRL) [7].

PAF is an unique phosphoglyceride, which possesses many potent biological activities relevant for various physiological and pathophysiological conditions [8–10]. Although originally described as an activator of platelets [4], PAF is now known to have a wide range of biological activities, acting on a long list of inflammatory cells that includes mast cells, mononuclear cells, neutrophils and eosinophils. In addition, PAF has potent biological effects on noninflammatory cells, such as smooth muscle cells, fibroblasts, endothelial cells, type II alveolar cells, and some neurologically derived cell lines.

Two principal pathways for the biosythesis of PAF have been discovered [11]. In the deacylation–reacylation pathway, PAF is derived from 1-O-alkyl-2-acyl-glycerophosphocholine cellular pools through the action of a phospholipase A_2. The resulting 1-O-alkyl-2-lyso-glycerophosphocholine is then acylated by an acetyl-CoA-requiring enzyme to form PAF [12]. Alternatively, PAF can be synthesized *via* a *de novo* pathway [13], which involves the transfer of phosphocholine from CDP-choline to 1-O-alkyl-2-acetylglycerol. The former pathway predominates in activated inflammatory cells, whereas the latter has been shown to be particularly important in a number of noninflammatory cells and isolated tissues.

Metabolic degradation of PAF occurs *via* an acetyl hydrolase, which removes the acetyl group leaving the molecule biologically inactive. The enzyme is associated with both low and high density lipoproteins and is present in blood, plasma or serum, as well as in various cells and tissues [11]. Removal of the acetyl group is a critical step, since other PAF metabolizing enzymes do not act on PAF directly. In view of its high biological activity, rapid inactivation of PAF may represent a vital physiological mechanism.

PAF comprises a family of multiple molecular species, including both saturated and unsaturated 1-O-alkyl homologues, 1-O-acyl analogues, and acetylated phosphoglycerides having polar head groups other than choline. Different molecular species of PAF can be produced by an inflammatory cell. The biological significance of the molecular heterogeneity is not yet clear. However, given the variable biological activity of each member of the PAF family [11], the inflammatory cell may be able to alter the predominate "type" of PAF, thereby determining the degree of inflammatory activity.

To complicate matters further, the molecular heterogeneity of PAF is met by the expression of apparently distinct PAF receptors. Several independent studies have proposed the existence of at least two distinct PAF receptor subtypes in platelets and leucocytes [14, 15]. HWANG [16] found that the relative potencies of PAF agonists and PAF antagonists on human platelets differ from those on neutrophils, and that the cellular responses to PAF in these cells could be differentiated by pertussis toxin, cholera toxin and the presence of monovalent cations Na^+ and Li^+. Furthermore, the expression of multiple receptor subtypes in the neutrophil [17] and the eosinophil [18] have been postulated, based on the presence of pertussis toxin-sensitive and pertussis toxin-insensitive PAF-dependent activity in each cell type. Binding studies identified a high affinity (Kd ≈ 0.2 nM for neutrophils, 0.3 nM for eosinophils) and a low affinity (Kd ≈ 200 nM and 11.5 nM, respectively) receptor on these leucocytes [17, 19]. More recently, microinjection of size-fractionated messenger ribonucleic acid (mRNA) from the promyelocytic leukemia cell line HL60 into the Xenopus oocyte showed that PAF receptor activity was broadly distributed in several mRNA fractions ranging from 3.5 to 6 kb [20]. This finding suggests that leucocytes may encode for distinct multiple PAF receptor subtypes.

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These results are in keeping with the recent molecular identification and cloning of the PAF receptor from guinea-pig lung by HONDA et al. [21]. Using a ligand specific cloning strategy, which involved the expression of complementary deoxyribonucleic acid (cDNA) on Xenopus oocytes, they demonstrated that the molecular architecture of the receptor shows homology to the G-protein-coupled receptors, with seven transmembrane spanning segments. The PAF receptor(s) is (are) linked to different intracellular pathways, including activation of glutamyl transpeptidase (GTPase), phospholipid turnover via phospholipases A_2, C, and D pathways, as well as activation of protein kinase C and tyrosine kinase. The fact that various ions can modulate the binding properties and affinities, the presence of heterogeneity in the PAF molecule itself and the recently noted oxidatively fragmented PAF molecule, all of which have PAF like activity, strongly support the view of an extremely complex mediator-receptor-effector interaction. PAF may not only use different molecular species, with different biological activities, but also different receptor subtypes, with various affinity states and different signalling mechanisms, to differentially regulate (patho)physiological processes, for instance in bronchial tissue in asthma.

Asthma is characterized by episodic, reversible airway narrowing, accompanied by an eosinophil-dominated inflammatory response and by persistent hyperresponsiveness of the airways, to a variety of stimuli. Various different inflammatory mediators, such as leukotrienes, prostaglandins or histamine, have been implicated in asthma. Although these mediators may account for some of the pathophysiological features of asthma, PAF has several properties which may be relevant to asthma, including effects on inflammatory cells, blood vessels, bronchial hyperresponsiveness, airway secretion, mucociliary transport, and bronchoconstriction [8, 9, 22].

PAF is recognized as being one of the most potent spasmogens both in experimental animals and man. In man, PAF is considerably more potent than established spasmogens, such as peptidoleukotrienes or histamine. In artificially ventilated humans suffering from cerebral death, intratracheal instillation of PAF caused bronchoconstriction [23]. In addition, normal subjects showed an immediate dose-dependent bronchoconstriction with 0.1 µmol PAF causing a 40% fall in the partial expiratory flow rate at 30% vital capacity [24]. However, no late-phase bronchoconstriction and no correlation with the responsiveness to methacholine was observed. In vitro, human airway only showed a variable contractile response to PAF in the presence of platelets and it failed to respond to PAF alone [25]. A dependency on platelets for bronchoconstriction was also reported in guinea-pig [26, 27] and rabbit [28], suggesting that PAF may exert its effects indirectly via releasing spasmogens from other cells, such as platelets.

The study by JOHNSON et al. [29] in this issue of the Journal examined eight specimens of bronchial tissue obtained from five patients resected at thoracotomy. Each specimen was exposed to PAF (700 nM) followed by methacholine (1 mM) and was then examined histologically. The authors conclude, in contrast to other studies cited above [25–28], that contraction of human isolated bronchus induced by PAF is not related to the release of mediators from secondary cells. Hence, the variability of the responses to PAF, observed in this study and previous studies [25, 30] may not be due to bronchial tissue dwelling cells but rather to a direct effect of the mediator.

What could be the reasons for these somewhat conflicting results? There are several important factors which the experimental design of the study did not control, thus weakening the findings of the study. Firstly, whilst no correlation was observed between leucocyte numbers and the contractile response, the activation of tissue dwelling cells and neuronal structures releasing spasmogenic mediators, such as prostaglandins [27], major basic protein (MBP) [31], tachykinins [32], or acetylcholine [33], cannot be ruled out. Hence, in order to strengthen the conclusions drawn from the experiments, it would have been interesting to evaluate the activation status of the cells. Using immunofluoresence techniques, this is a comparatively easy procedure. Activation of the eosinophil, for instance, can be determined using EG1 or EG2 antibodies [34] or by staining for CD11b [35]. In addition, transmission electron microscopy could have been used to evaluate the state of the granularity of the cells, such as the eosinophil [36].

Secondly, whilst neutrophils, eosinophils, lymphocytes, plasma cells and epithelium were included in the study, macrophages, basophils and mast cells were not accounted for. Since basophils and macrophages can be activated by PAF, the smooth muscle contraction observed may be due to the release of histamine or other biologically active mediators. Although there is no evidence as yet that mast cells can be activated by PAF directly [37], it is conceivable that PAF may induce the secretion of tachykinins, which in turn could stimulate the mast cells to secrete histamine and other mediators.

Thirdly, a possible way of evaluating whether PAF has a direct effect on smooth muscle cells, would be to expose human bronchial tissue to stimuli such as immunoglobulin E (IgE) or formyl-methionyl-leucyl-phenylalanine (fMLP) that activate inflammatory tissue dwelling cells but not smooth muscle cells in the presence of a PAF receptor antagonist. A response of smooth muscle to such a treatment would not only rule out a direct effect of PAF but clearly implicate other mediators in bronchoconstriction.

Finally, the concentration close to 1 µM, used to induce a contractile response of the bronchial tissue, is comparatively high and a nonspecific or (sub)toxic effect was not excluded by JOHNSON et al. [29]. It might have been helpful to employ PAF receptor antagonists in order to exclude this effect. Furthermore, since PAF receptors may exist in different affinity states, it would be interesting to know whether the bronchial smooth muscle contracts in a concentration-dependent manner.

Therefore, does airway smooth muscle cell seriously care about PAF? Clearly, as outlined here, there is a considerable body of evidence that PAF is "only" acting upon airway smooth muscle cells indirectly, *via* the release of bronchoconstricting mediators from secondary cells. However, given the considerable heterogeneity of PAF molecules, PAF receptors and transmembrane pathways, the possibility that other, as yet less established, mechanisms may be involved, has to be considered. For instance, in keeping with the effects of PAF on other cells or tissue [8–10], higher *in vitro* concentrations used in this study may elicit a direct effect, whilst lower concentrations may not. The heterogeneity or PAF may also help to explain another, as yet unanswered, question relating to biological variability among asthmatic subjects.

Clearly, more studies are needed before the conclusions drawn by JOHNSON *et al.* [29, 30] can be fully accepted. Whether or not airway smooth muscle is affected by PAF directly, the potential significance of PAF remains. However, given the number and variety of inflammatory mediators, it seems unlikely that any single mediator accounts for all pathophysiological processes associated with bronchoconstriction.

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