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# COMMENTARY

# FROM ANTI-ASTHMA DRUGS TO PAF-ACETHER ANTAGONISM AND BACK

## PRESENT STATUS

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Two mechanisms prevail in the pathophysiology of asthma: (1) bronchial smooth muscle constriction, which accounts essentially for its early phase, and (2) inflammation of the airways, rather associated to the late phase of asthma and characterized by epithelial damage, excessive mucus production, bronchial edema and accumulation of inflammatory cells in the bronchial wall. Inflammatory mediators participate in both phases (Fig. 1). In addition, recent developments indicate that the mechanisms accounting for inflammation (chemical and cellular mediation) cannot be understood fully without considering the role of the nervous system for the control of bronchial tone, of vasopermeability and of inflammation. Besides the recognized role of the sympathetic and parasympathetic nervous systems, a non-adrenergic non-cholinergic system is now recognized but, at this stage, very few studies have been performed to correlate it with chemical mediation and particularly with the phospholipid mediator, 1-alkyl-2-acetyl-*sn*-phosphoryl-3-choline (PAF-acether). In this commentary, we shall discuss present developments concerning the role of PAF-acether in bronchial asthma, with particular emphasis on the different antagonists recently described.

### REGULATORY SYSTEMS OF AIRWAY RESPONSIVENESS

#### *Parasympathetic system*

Post-ganglionic parasympathetic fibers innerve the bronchial glands, the pulmonary vessels, and the smooth muscle up to the distal area of the terminal bronchioles [1]. An enhanced cholinergic activity of the airways of asthmatic patients, associated with several other mechanisms, has been described, including: (i) an increased frequency of the discharges of the receptors from afferent irritants and C fibers, (ii) the exposure of afferent nerve endings following epithelium damage; (iii) an increase of the number or of the affinity of muscarinic receptors [2], or (iv) an enhanced responsiveness of smooth muscle to cholinergic agonists [2].

#### *Sympathetic system*

Sympathetic fibers regulate pulmonary vascular and bronchial tone [3], and reduced levels of catecholamines in blood of asthmatic patients, which failed to increase during exacerbations or stress [2], were demonstrated.

A reduced cell response to $\beta$-adrenergic stimuli [4] or a decrease in the number of $\beta$-adrenergic receptors [5] during antigen- or exercise-induced asthma [6] was described, even though comparable amounts of cAMP are released following $\beta$-receptor stimulation in white blood cells [7] from asthmatics as from healthy subjects. Furthermore, a similar number of $\beta$-receptors on cells from atopic and non-atopic patients was shown [8]. The decrease of $\beta$-receptors may thus result from tolerance after treatment with $\beta$-agonists [2, 7] or from the release of inflammatory mediators [2]. It has been claimed recently that exposure of human lung parenchyma strips to PAF-acether decreases the number of $\beta$-adrenoceptors, although only minimal alterations of the relaxant responses to isoproterenol were noted [9]. In contrast, Barnes *et al.* [10] found no difference in the number and affinity of $\beta$-receptors in lung tissue following administration of PAF-acether, whereas a loss of sensitivity to isoproterenol was noted, which may result from the airway obstruction secondary to cell infiltration and edema following PAF-acether administration. Criscuoli and Subissi [11] showed that pretreatment with $\beta$-antagonists decreases the survival of mice injected with PAF-acether. Interestingly, $\beta_2$-receptor agonists and dexamethasone prevented PAF-acether-induced death, whereas cyclooxygenase inhibitors and peptido-leukotriene antagonists were ineffective.

#### *Non-adrenergic non-cholinergic system (NANC system)*

*Non-adrenergic system.* This system, formed by preganglionic fibers, predominates in large airways [12] and may account for the relaxation of the airway smooth muscle [2, 13] and for the production of airway mucus after vagal [14] or electrical field stimulation [15].

Vasointestinal peptide (VIP), one of the neurotransmitters involved with this system, relaxes bronchial smooth muscles of different species, including humans. VIP is found in lung neurons, nerve terminals, airway smooth muscle, in sub-mucosal glands and in bronchial and pulmonary vessels [2, 16] of

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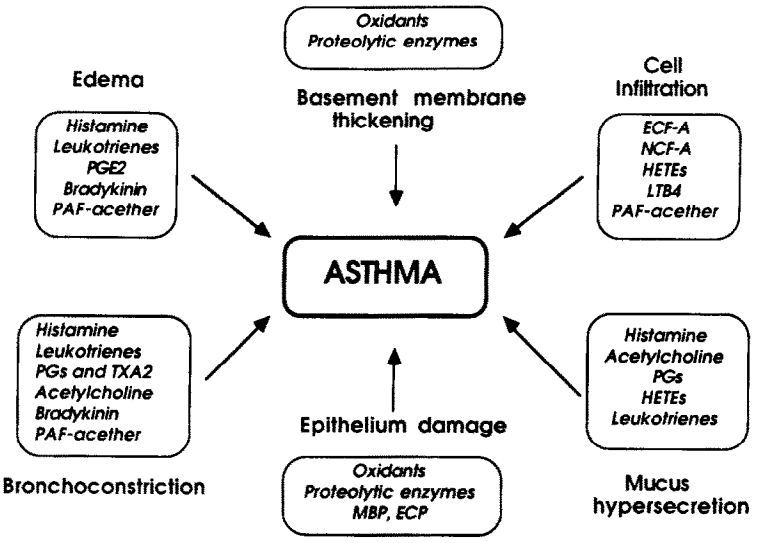


Fig. 1. Participation of various mediators in bronchopulmonary alterations characterizing asthmatic reactions. Abbreviations: ECF-A, eosinophil chemotactic factor; NCF-A, neutrophil chemotactic factor; HETE, monohydroxyeicosatetranoic acid; $LTB_4$, leukotriene $B_4$; PAF-acether, platelet-activating factor; PGs, prostaglandins; MBP, major basic protein; ECP, eosinophil cationic protein; and $TXA_2$, thromboxane $A_2$.

animals and humans. It is fifty times as effective as isoproterenol [17] and prevents histamine- and serotonin-induced bronchoconstriction [18]. VIP impairs histamine release induced by antigen challenge in isolated chopped lungs from sensitized guinea pigs [19]. Recently, Di Marzo *et al.* [20] demonstrated that the release of peptido-leukotrienes from guinea pig lungs induced by PAF-acether is inhibited markedly by VIP, reinforcing its hypothetized regulatory role for the control of the bronchopulmonary function.

*Non-cholinergic system.* The neutrotransmitters regulating the function of this system are the tachykinines, including substance P (SP), a potent bronchoconstrictor agent in guinea pigs [21]. Bronchoconstriction by SP is accompanied by an increased microvascular permeability and by edema of the airway walls. A marked cardiovascular effect associated with a mild bronchoconstriction followed by bronchodilatation was noted in humans [22]. SP promotes airways mucus secretion in dogs [2] and induces the release of mediators from peritoneal rat mast cells and from human skin [2, 23]. Recently, it has been proposed that SP plays a critical role in enhancing neutrophil and eosinophil responses to chemotactic agents [24].

A relationship between the effects of PAF-acether and the control of lung function by SP has been proposed recently by Rodrigue *et al.* [25], who noted that SP is released when guinea pig chopped bronchi are incubated with PAF-acether.

## PAF-ACETHER AND THE PATHOPHYSIOLOGY OF EXPERIMENTAL ASTHMA

The involvement of PAF-acether in IgE-dependent anaphylaxis in the rabbit was first reported by Henson and Pinckard [26] who demonstrated that acether after systemic anaphylaxis, play a crucial role for antigen- and PAF-acether-induced bronchopulmonary alterations [27]. In 1980, Vargaftig *et al.* [28] further emphasized the resemblance between anaphylactic shock and the effects of PAF-acether in the guinea pig, demonstrating that platelets are needed for bronchoconstriction (but not for hypotension), and studied potential inhibitors [29]. Platelet secretion is critical for the bronchopulmonary effects of PAF-acether in the guinea pig, since drugs which prevent platelet activation such as prostacyclin or sulphinpyrazone [30], or which block the effects of platelet-derived mediators [29], impair bronchoconstriction by PAF-acether. The direct proof of the platelet involvement with the bronchopulmonary effects of PAF-acether was obtained when platelet-depleted animals [28] were shown not to undergo bronchoconstriction following its intravenous injection. Neutrophil depletion fails to interfere with bronchoconstriction by PAF-acether.

Bronchoconstriction by PAF-acether has also been shown in various animal species (reviewed in Ref. 31). The aerosolization of PAF-acether to healthy humans resulted in bronchoconstriction and a long lasting bronchial hyperreactivity to subsequent stimulation with methacholine [32].

Further evidence implicating PAF-acether in anaphylactic reactions in guinea pigs was obtained with specific antagonists (Table 1). However, their effects are markedly dependent on the protocol of immunization and on the route of antigen administration. Thus, Lagente *et al.* [33] and Pretolani *et al.* [34] demonstrated that two chemically unrelated PAF-acether antagonists, BN 52021 and WEB 2086, inhibit the bronchopulmonary and hematological alterations following the intravenous injection of antigen to guinea pigs passively sensitized with

Table 1. References to comparative effects of various antagonists on the bronchopulmonary and skin alterations induced by PAF-acether and antigen

| Biological effects evoked by: | PAF-acether | Antigen |
|---|---|---|
| | References | |
| Lung | | |
| Bronchoconstriction | [34, 37, 126] | [31, 33, 34, 37, 126, 137] |
| Thrombopenia | [31, 34, 126, 138, 139] | |
| Leukopenia | [31, 34, 37, 126] | [31, 33, 34] |
| Cell infiltration | [64, 127] | [64, 127, 128, 131] |
| Increase of vascular permeability | [89, 140] | |
| Bronchial hyperreactivity | [100, 127] | [127, 128, 141] |
| Mediator release from isolated perfused lung | [34, 126, 142] | [33, 34, 143] |
| Skin | | |
| Wheal and flare | [129, 131] | [130] |

ive transfer of anti-ovalbumin rabbit serum, BN 52021 (reviewed in Ref. 31) and WEB 2086 [35] significantly reduced antigen-induced bronchoconstriction and, to some extent, the associated thrombocytopenia. Higher inhibition was reached when the antagonists were associated with an antihistamine drug. Inhibition by BN 52021 of antigen-induced bronchoconstriction was reported in guinea pigs immunized with high amounts of ovalbumin (reviewed in Ref. 31). In contrast, BN 52021 (Desquand *et al.*, unpublished observations*) and WEB 2086 [34] were inactive against bronchopulmonary alterations in animals actively sensitized with a protocol using low amounts of ovalbumin. These differences in the pharmacological control of active anaphylaxis by PAF-acether antagonists may involve not only the immunoglobulin classes concerned, but also the components of the immune system (lymphocytes and accessory cells) implicated in the sensitization process.

The pharmacological modulation of the anaphylactic shock is more complex when the intravenous route is used for the administration of antigen, and its antagonism requires a "cocktail" of drugs [36], probably because of the rapid and massive release of various mediators from different cell types and organs. The difficulty in suppressing the anaphylactic shock following the intravenous route was by-passed by administering the antigen by aerosol. In this case, bronchoconstriction is not accompanied by thrombocytopenia or leukopenia [37]. By contrast, resident pulmonary cells such as macrophages, mast cells or other inflammatory cells which might have migrated into the lungs (neutrophils, eosinophils and platelets) may constitute major targets for the antigen. Corticosteroids, $\beta$-agonists, or PAF-acether antagonists [37] are more effective in blocking bronchoconstriction against aerosolized antigen than against its systemic administration.

The reactivity of lungs from actively sensitized guinea pigs to PAF-acether, leukotriene (LT) $D_4$, histamine and arachidonic acid is increased as compared to those from non-sensitized or passively sensitized animals [38]. These changes in lung reactivity following immunization remain presently unexplained, although the implication of immunocompetent cells is likely, since the alterations of reactivity are fully dependent upon the booster injection of the antigen and persist for a longer period of time than circulating homocytotropic antibodies [38].

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## INVOLVEMENT OF INFLAMMATORY CELLS

Several cell types can generate PAF-acether when challenged with appropriate stimuli. These cell types include basophils [39, 40], alveolar macrophages [41], polymorphonuclear neutrophils (PMN) [42] and eosinophils [43], platelets [44], mast cells [45] and endothelial cells [46]. In addition, most of these cells not only generate PAF-acether, but also are targets for this mediator.

### *Basophils*

Basophils, which share morphologic and functional similarities with mast cells, contain histamine, associated to a matrix of proteoglycans [47], and express a high number of high-affinity IgE receptors ($Fc_\varepsilon RI$) [48]. IgE-sensitized rabbit basophils release histamine and a soluble mediator which induces the secretion of histamine from platelets upon antigen or anti-IgE stimulation [39]. This, and the fact that platelet aggregates are formed at the vicinity of degranulated basophils when they are co-suspended and stimulated with antigen, led to the hypothesis that PAF-acether was generated by basophils [39]. Basophils also secrete histamine [47], prostaglandin (PG) $D_2$ [49] and $LTC_4$ [40]. However, since these cells have never been identified in the airways of asthmatic patients, their direct implication for acute asthma remains to be established.

### *Alveolar macrophages*

Alveolar macrophages possess a low affinity receptor ($Fc_\varepsilon R2$) for IgE [50] and release inflammatory mediators upon stimulation with the specific antigen: thromboxane (TX) $A_2$, leukotrienes, and prostaglandins (reviewed in Ref. 51). In addition, rabbit, rat and human alveolar macrophages generate PAF-acether when stimulated with the calcium ionophore A23187, whereas the release of the phospholipid mediator appears to be species dependent when these cells are stimulated with zymosan particles (reviewed in Ref. 51). Alveolar macrophages also can be activated by these mediators when they are released by other cell types during the allergic reaction [52].

Alveolar macrophages respond to PAF-acether by producing prostaglandins and superoxide anions [53]. By contrast, *in vitro* challenge of alveolar macrophages from actively sensitized guinea pigs with antigen is not followed by activation, whereas alveolar macrophages from allergic human subjects respond to allergen administration with the formation of superoxide anions [50]. In one report, alveolar macrophages from allergic subjects were shown to release PAF-acether when stimulated *in vitro* with the specific antigen [52]. This further emphasizes the differences between the alveolar macrophages according to their source. Recently, however, the possibility that alveolar macrophages may play a role in the lung responses to the antigen in the guinea pig has been raised, since it was demonstrated that PAF-acether reduces the increase in cyclic AMP content of guinea pig alveolar cell population evoked by $PGE_2$, salbutamol or isoproterenol [54]. Furthermore, the cyclic AMP content of alveolar macrophages from actively sensitized guinea pigs is markedly less augmented by $PGE_2$ or salbutamol as compared to those from non-sensitized animals [54], suggesting that the immunization of the animals leads to a cell defect.

*Polymorphonuclear neutrophils (PMN)*

PMN are also potential targets for PAF-acether. Indeed, rabbit and human PMN stimulated with PAF-acether generate lipoxygenase metabolites of arachidonic acid, including 5-hydroxy-eicosatetraenoic acid and $LTB_4$ (reviewed in Ref. 55). In addition, PAF-acether induces lysosomal enzyme release, chemotaxis and superoxide anion production by human PMN and increases that evoked by other agonists (reviewed in Ref. 55). PMN generate PAF-acether when stimulated with ionophore A23187 or with opsonized zymosan [42]. Interestingly, the release of PAF-acether by human PMN is enhanced by lyso-PAF, which can be explained by the high levels of acetyltransferase present in this cell type [56]. The production of PAF-acether by PMN may be a critical step for the bronchopulmonary alterations observed during asthmatic reactions, since PMN are recruited into lungs during the late phase (reviewed in Ref. 57).

*Eosinophils*

Eosinophils are attracted and activated essentially by three important chemotactic substances: ECF-A, $LTB_4$ and PAF-acether. Their response to these agonists, as well as their secretory capability, depends upon their state of activation. Two types of eosinophils have been defined according to their density: normodense and hypodense cells, which differ functionally and morphologically [58]. The hypodense eosinophils are more active and are usually present in subjects with eosinophilia from various origins, including asthma. The normodense eosinophils are present in healthy and hypereosinophilic subjects (reviewed in Ref. 59). Eosinophils express receptors for various complement components (CR1 and CR3) [60] and for IgG [59] and IgE [61]. The eosinophil IgE Fc receptors ($Fc_\varepsilon R2$) [61] display an affinity lower than that of IgE mast cell receptors ($Fc_\varepsilon R1$) (reviewed in Ref. 59).

Additional proof for the involvement of eosinophils in allergic reactions was provided by Henocq and Vargaftig [62], who compared the cell response during cutaneous inflammation following the administration of PAF-acether, or the chemotactic and secretagogue peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), or allergen to atopic and non-atopic subjects. These studies demonstrated that PAF-acether and fMLP are more effective in eliciting *in vivo* eosinophil degranulation in atopic patients as compared to controls. Moreover, the number of eosinophils found in the site of acute reaction is higher in atopic subjects, whereas in normal individuals they are practically not observed, the majority of cells being neutrophils. This suggests that eosinophil responsiveness is qualitatively different when they originate from atopic patients and that PAF-acether and fMLP not only can activate the cells involved in allergic reactions, but also can enhance the inflammatory response. A qualitative difference in the response of eosinophils from asthmatic patients to PAF-acether was also shown by Borgeat *et al.* [63], who demonstrated that the mediator induces an enhanced release of $LTD_4$ as compared to those from non-atopic patients, an effect suppressed by a specific PAF-acether antagonist [63].

Eosinophil recruitment into the guinea pig lung parenchyma and bronchial sub-mucosa following intravenous or aerosol administration of PAF-acether was also demonstrated [64]. Interestingly, aerosolization or the intravenous administration of the antigen to sensitized animals is also followed by leukocyte aggregation and massive infiltration of eosinophils [64]. These results further strengthen the analogy between the effects of PAF-acether and of antigen on the lung function. Recently, Lellouch-Tubiana *et al.* [64] reported that two PAF-acether antagonists, BN 52021 and WEB 2086, as well as prostacyclin and immune platelet depletion, impair eosinophil infiltration following antigen challenge to passively sensitized guinea pigs. This further supports a role for PAF-acether in cell recruitment at the site of allergic reactions and suggests, in agreement with Morley *et al.* [65], that platelets are involved in the bronchopulmonary alterations of allergic reactions.

*Platelets*

The initial description of the biological activity of PAF-acether was based on its effect on platelets. Indeed, PAF-acether aggregates human, rabbit, guinea pig and dog platelets (reviewed in Ref. 66). In contrast, rat and mouse platelets are refractory to the mediator *in vitro* [67], even though the infusion of PAF-acether to rats evokes a late thrombocytopenia [68], possibly related to the generation of secondary mediators. PAF-acether also induces platelet secretion of $TXA_2$ (although to a much lower extent than ionophore A23187, thrombin or collagen), platelet factor 4, platelet-derived growth factor, ADP and serotonin and/or histamine (reviewed in Ref. 66). Moreover, aggregation of human platelets induced by adrenaline is potentiated by PAF-acether, which also regulates the expression of the gly-

coproteins IIb/IIIa, the platelet fibrinogen receptor (reviewed in Ref. 66). The presence of degranulated platelets at the proximity of target respiratory muscle cells after PAF-acether or antigen administration [64] agrees with suggestions that platelets may be early targets for PAF-acether and antigen in acute allergic bronchoconstriction [28, 29, 65].

*Mast cells*

Mast cells are present in the airways and between the basement membrane and bronchial epithelium and possess 80–300 metachromatic granules containing a large variety of preformed pro-inflammatory mediators [69]. During specific or non-specific stimulation, mast cells release their granular content and synthesize newly formed mediators, including arachidonic acid metabolites [70]. Although murine serosal mast cells do not generate PAF-acether when stimulated [41], those differentiated from bone marrow precursors in the presence of T-cell growth factor(s) produce the phospholipid mediator upon antigen challenge [71]. Isolated human lung mast cells form PAF-acether without releasing the autacoid into the incubation medium [72]. Human endothelial cells also generate PAF-acether, which remains cell-associated [46], suggesting its participation in cell-to-cell interactions.

The release of the various mast cell mediators correlates with the severity of asthma and bronchial hyperreactivity [73]. Thus, this cell type could play an important role in the early and, possibly, the late phase of asthma [73–75].

*Endothelial cells*

Endothelial cells stimulated with PAF-acether mobilize calcium and contract in response to the mediator [76]. The effect of PAF-acether on endothelial cells may explain the vasopermeant effect of the phospholipid in human, guinea pig and rat skin, which is accompanied by platelet accumulation (reviewed in Ref. 55). By contrast, the increase in vascular permeability induced by PAF-acether is not platelet-mediated in rats, rabbits or guinea pigs (reviewed in Ref. 55). Interestingly, when PAF-acether is injected intradermally into the rat, vascular lesions and thrombus formation are observed, even though rat platelets are refractory to its direct effect. Recently, Bourgain *et al.* [77] demonstrated that topical administration of PAF-acether onto the guinea pig mesenteric artery is followed by endothelium alterations and thrombus formation. The PAF-acether precursor and metabolite lyso-PAF had no pro-thrombotic activity, unless it was associated to a sub-effective electrical challenge, suggesting that the latter releases and/or activates acetyltransferase from vascular endothelium, with a consequent formation of PAF-acether. This interpretation is supported by the fact that PAF-acether antagonists block altogether the effects of PAF-acether itself and of lyso-PAF. Furthermore, the anti-asthmatic drug ketotifen, which is claimed to interfere with acetyltransferase activity [78], suppresses the effects of lyso-PAF only, whereas those of PAF-acether are unmodified. It is thus likely that under appropriate conditions, particularly at the proximity of injured cells, lyso-PAF is reconverted directly into PAF-acether, thus reinforcing inflammation.

*Airway epithelium*

The protective effect of the epithelium is reduced in asthma [79], leading to an enhanced passage of antigen(s) to the target cells below the basal membrane. The epithelial lesions of the disease may expose C fiber endings to unspecific stimuli; furthermore, since the epithelium is involved with particle clearance, its alterations are followed by the accumulation of mucus plugs. In addition, mediators are formed by the epithelium: $PGE_2$ in rats [80] and guinea pigs ([81], Nahori *et al.*, unpublished observations*), and lipoxygenase derivatives in dogs [82] and humans [83]. A relaxant effect of PAF-acether on isolated guinea pig trachea was reported and appears not to be mediated by endogenous prostaglandin formation [84]. Brunelleschi *et al.* [85] described a similar *in vitro* relaxation accompanied by increased levels of $PGE_2$ which was suppressed by indomethacin, suggesting that PAF-acether relaxes tracheal strip preparations by a mechanism involving the epithelium cyclooxygenase pathway.

Platelets, neutrophils and eosinophils may mediate the bronchopulmonary alterations of asthma. In the guinea pig, intravenous injections of PAF-acether induce platelet and neutrophil sequestration in the lungs [86, 87], as well as an increase in extravascular albumin [87]. Platelet accumulation is not restricted to PAF-acether, since other platelet activators such as ADP, collagen and the thromboxane mimetic U-46619 also induce pulmonary platelet recruitment [88], whereas only PAF-acether induces subsequent bronchopulmonary hyperreactivity, which is thus not accounted for by platelet recruitment alone. It was suggested that the interaction of PAF-acether with platelets leading to bronchopulmonary hyperreactivity is secondary to the activation of other cell types, such as neutrophils and eosinophils and/or to the ability of the phospholipid to affect vascular endothelium [46] and to evoke edema of the airways [89]. Therefore, following intravenous injections of PAF-acether, electron microscopy observations uncover marked degenerative lesions of the bronchial epithelium associated with platelet margination and degranulation within lung tissue. Furthermore, platelet margination is the first event following intravenous injections of PAF-acether to the guinea pig [64]. Then, neutrophils and eosinophils are recruited and appear degranulated [64]. Interestingly, Bourgain *et al.* described ultrastructural analogies between PAF-acether-induced thrombosis in the guinea pig mesentery [90] and the pulmonary effects. Indeed, the series of events which characterize the thrombus formation starts with platelet adhesion, is followed by leukocyte migration, and then by a late-phase in which eosinophils are activated.

## PHARMACOLOGICAL CONTROL OF ASTHMATIC REACTIONS

*Steroidal anti-inflammatory drugs*

Glucocorticosteroids are the most potent anti-

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inflammatory drugs employed in the treatment of the early and the late phase of asthma [91]. After entering the cell by diffusion, glucocorticosteroids bind to a cytoplasmic receptor; the activated complex is translocated to the nucleus where it interacts with desoxyribonucleic acid and chromatin, leading to the synthesis of mRNA and of mediator proteins [92]. An alternative non-genomic mechanism has also been suggested [92].

*Interference with asthma.* Glucocorticosteroids are said to restore or enhance the sensitivity of β-adrenergic receptors to catecholamines *in vivo* [93] and *in vitro* (reviewed in Ref. 94). Hydrocortisone increases the number of β-receptors in rat lung, cultured human lung cells and human leukocytes (reviewed in Ref. 94). One of the most important effects of glucocorticosteroids is to inhibit the release of arachidonic acid [95] by inducing the synthesis of lipocortins, a family of proteins that blocks phospholipase $A_2$ activity [96] and, accordingly, the release of eicosanoids and leukocyte chemotaxis (reviewed in Ref. 94). The protective effect of glucocorticosteroids during the late phase reaction following antigen administration may result from their blockade. In addition, these drugs inhibit the mucus bronchial secretion [97] and accelerate its clearance [98]. Furthermore, corticosteroids decrease the number of IgE receptors in medullary and in bone-marrow derived mast cells [99].

It has been claimed that bronchoconstriction by PAF-acether in the guinea pig is inhibited by hydrocortisone [100]. Furthermore, Chignard *et al.* [101] have shown that the formation of PAF-acether by guinea pig lungs upon *in vitro* antigen challenge is reduced markedly by the glucocorticosteroid budesonide. Since the latter also inhibits the IgE-mediated anaphylactic bronchoconstriction in actively sensitized guinea pigs [102], these results further support a role of PAF-acether as mediator of bronchopulmonary anaphylaxis. It is noteworthy that the glucocorticosteroid dexamethasone failed to block bronchoconstriction induced by antigen administered systemically to actively sensitized guinea pigs (unpublished observations), possibly because of the overwhelming amounts of histamine released from the liver and other sites.

Other effects of glucocorticosteroids which can contribute to their anti-asthmatic activity include suppression of the production of lymphokines and monokines (reviewed in Ref. 59), decrease of the release of eosinophil cationic protein [103] and suppression of eosinophil colony formation in peripheral blood (reviewed in Ref. 59), decrease of the release of neutrophil chemotactic factor and inhibition of neutrophil activation [104], and increase of neutrophil colony formation in peripheral blood (reviewed in Ref. 59).

*Disodium cromoglycate (DSCG) and nedocromil sodium*

DSCG inhibits the early and the late phase of antigen-induced IgE-dependent reactions [105], reduces bronchial hyperreactivity which follows antigen provocation in asthmatics [106] and antigen-mediated basophil degranulation, and increased vasopermeation during cutaneous anaphylaxis in guinea pigs [107]. Since DSCG inhibits the release of histamine and leukotrienes by antigen-stimulated human lung fragments [108], it has been proposed that it stabilizes the mast cell and the alveolar macrophage membrane [109, 110]. Stabilization may involve the blockage of $Ca^{2+}$ transport following the phosphorylation of a 78 kD protein [111]. A direct role of membrane stabilization is challenged by the demonstration that β-adrenoceptor agonists, which are more potent than DSCG in stabilizing the mast cell membrane, fail to block the late phase reaction of asthma (reviewed in Ref. 112).

Since DSCG inhibits the permeability of airway epithelial cells and plasma exudation in the guinea pig bronchial lumen [113], a role for a specific anti-inflammatory effect can be claimed. Indeed, the delayed skin reactions to allergens in atopic subjects, which are said to correspond to the late-onset responses after allergen inhalation [114], are blocked by very high concentrations of intradermal cromoglycate [115].

Day *et al.* [116] demonstrated a marked delayed response when supernatant fractions from activated platelets (but not from leukocytes) were injected intradermally into humans. A role for PAF-acether is further supported by the observation that its intratracheal instillation to rabbits triggers a late-onset response which is blocked by DSCG [65]. Finally, a protective action of DSCG against capsaicin-induced bronchoconstriction [117] and bronchopulmonary alterations evoked by neuropeptides in rats [118] has been reported. Nevertheless, administered i.v. to the guinea pig, neither cromoglycate nor nedocromil sodium (which belongs to the same chemical family as DSCG) interferes with the acute effects of PAF-acether, such as bronchoconstriction, hypotension, leukopenia or thrombocytopenia [119]. Nedocromil sodium also fails to prevent release of histamine and of $TXB_2$ induced by antigen administration to isolated lungs obtained from actively sensitized guinea pigs.

*Ketotifen*

Ketotifen prevents PAF-acether-induced bronchopulmonary hyperreactivity in the guinea pig [100] and, at high doses which antagonize other agents as well, blocks the acute bronchoconstrictor effects of PAF-acether [120]. Joly *et al.* [78] demonstrated that release of PAF-acether and acetyltransferase activity of IgE-sensitized bone marrow-derived mast cells stimulated with the specific antigen are blocked by ketotifen. This agrees with findings that the latter inhibits the influx of calcium ions following the depolarization of the smooth muscle cell membrane [121]. In addition, Bourgain *et al.* [77] showed that thrombosis induced by the association of a sub-effective electrical stimulus and lyso-PAF applied topically onto the guinea pig mesenteric artery is antagonized by ketotifen, supporting an interference with the conversion of lyso-PAF into PAF-acether, probably by inhibition of acetyltransferase activity. Ketotifen, nevertheless, does not inhibit the activity of purified acetyltransferase (Morley, personal communication*), but should rather inhibit its activation.

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*Drugs acting via the cyclic AMP system*

Drugs which augment the intracellular concentrations of cyclic AMP, i.e. $PGE_2$, theophylline and $\beta_2$ agonists, prevent acute bronchoconstriction by systemic PAF-acether (reviewed in Ref. 31). Inhibition by prostacyclin of bronchoconstriction by systemic PAF-acether in the guinea pig [30] is mediated by its platelet-protective effect. Indeed, as stated above, bronchoconstriction by i.v. PAF-acether is platelet-dependent [28]. It is common knowledge that the increase in cyclic AMP content prevents activation of inflammatory cells; accordingly, the drugs effective in this system also reduce acute anaphylaxis.

## PAF-ACETHER ANTAGONISTS

The introduction of PAF-acether antagonists was essential for unravelling its participation in a large variety of physiopathological situations. They can be classified in two main groups, according to their chemical structure.

(1) *PAF-acether related antagonists*

The first compound described in this series was CV 3988 [122], which inhibits PAF-acether-induced platelet aggregation and the subsequent production of phosphoinositides from platelet membranes [123]. However, at high concentrations, CV 3988 also blocks arachidonic-acid-, ADP-, collagen- and ionophore A23187-induced platelet aggregation (reviewed in Ref. 31). *In vivo*, CV 3988 protects against PAF-acether-induced bronchopulmonary and hematological alterations and antagonizes endotoxic shock in the rat (reviewed in Ref. 31).

The structurally related PAF-acether antagonist Ro 19-3704, the most potent out of a series, inhibits bronchoconstriction by PAF-acether itself and by aerosolized antigen in guinea pigs passively sensitized with homologous serum [37]. Other structurally related compounds, such as SRI 63-119 and SRI 63-072, interfere with PAF-acether-induced bronchoconstriction and hypotension in the rat and in the guinea pig [124]. Ro 19-3704 and its analogs also block epinephrine-induced aggregation of human platelets, in contrast to the standard PAF-acether antagonists BN 52021 or WEB 2086 which are completely inactive (reviewed in Ref. 31).

(2) *PAF-acether unrelated antagonists*

*Natural products: terpenes.* The ginkgolides A, B, C and M (BN 52021, BN 52022, BN 52023 and BN 52024, respectively), isolated from the Chinese tree *Ginkgo biloba* were found to be PAF-acether antagonists [125]. Since their properties were reviewed elsewhere [31], we shall briefly consider their relevance for experimental asthma and the preliminary results obtained in humans.

As stated above, BN 52021 inhibits dose-dependently PAF-acether-induced bronchoconstriction and the associated thrombocytopenia [126]. BN 52021 also inhibits the development of bronchial hyperreactivity induced by PAF-acether in the guinea pig and prevents antigen-induced bronchopulmonary hyperreactivity in the guinea pig [127] and in the rabbit [128]. BN 52021 blocks antigen-induced bronchoconstriction in passively sensitized guinea pigs, both in an homologous system as well as in an heterologous system, in which immune rabbit plasma was transferred to guinea pigs (reviewed in Ref. 31).

The ginkgolide mixture BN 52063 (BN 52020, BN 52021 and BN 52022, in a weight ratio of 2:2:1) has been used in a limited series of clinical trials. BN 52063 markedly decreases the wheal and flare reactions induced by intradermal injections of PAF-acether in healthy subjects [129]. Interestingly, oral administration of BN 52063 inhibits the wheal (but not the flare) response and the late reaction induced by the allergen in human skin [130]. Recently, Guinot *et al.* [131] demonstrated that BN 52063 reduces the hyperresponsiveness to methacholine observed 6 hr following allergen provocation. Chung and Barnes [132] failed to demonstrate a protective effect of this drug on PAF-acether-induced bronchoconstriction and the associated neutropenia in humans. This may indicate that BN 52063 does not reach the airways epithelium and agrees with animal results showing that bronchoconstriction induced by PAF-acether aerosolized to non-sensitized guinea pigs primarily involves cyclooxygenase metabolites [29].

*Synthetic products: triazolobenzodiazepines (TBDZ).* Kornecki *et al.* [133] demonstrated that alprazolam and triazolam inhibit PAF-acether-induced human platelet activation. Brotizolam inhibits the effects of PAF-acether in various experimental models *in vitro* and *in vivo* (reviewed in Ref. 31), suggesting a possible relationship between the PAF-acether antagonism and the effects on central nervous system. However, works by Casals-Stenzel and Weber [134] (reviewed in Ref. 31), ruled out this hypothesis, demonstrating that a specific TBDZ antagonist blocks the effects of TBDZ on the central nervous system without interfering with brotizolam-induced PAF-acether inhibition. The search for TBDZ devoid of hypnogenic activity led to the synthesis of WEB 2086, a compound which, besides its potent and specific PAF-acether antagonistic activity, is effective against various models of anaphylaxis [34, 35] and endotoxin-induced shock [135]. It was demonstrated recently that WEB 2086 given by aerosol, by the intravenous or the oral route to healthy volunteers, inhibits *ex vivo* PAF-acether-induced platelet aggregation [136].

## PAF-ACETHER AND EXPERIMENTAL ASTHMA: CONCLUDING REMARKS

Given the limited number of studies available, the therapeutic effects of PAF-acether antagonists remain to be ascertained. However, it seems already likely that this new class of drugs will primarily affect the late rather than the early phase of the allergic reaction. Furthermore, failure of drug associations [28] or of selective PAF-acether antagonists such as BN 52021, WEB 2086 or Ro 19-3704 to suppress acute bronchoconstriction of active anaphylactic shock to the same extent as a trivial antihistamine, in contrast with their effectiveness against passive shock, particularly when triggered by antigen aero-

solization, should be understood. Active shock involves multiple mechanisms, mediators and targets, some of which are PAF-acether independent. Thus, recent results show that bronchoconstriction triggered by antigen aerosolized to guinea pigs sensitized by a single injection of antigen is more readily inhibited by BN 52021, whereas the booster injection of antigen makes bronchoconstriction more resistant to the PAF-acether antagonist. Furthermore, the possible existence of distinct binding sites for PAF-acether and of different molecular species further complicates the issue and may account for difficulties in finding anti-allergic antagonists based on tests performed against PAF-acether-induced platelet activation only.

Therefore, whether PAF-acether antagonists will or will not show useful anti-bronchoconstrictor or anti-exudative effects should not alter the concept that they may be used to control the recruitment into lungs of inflammatory cells, which aggravate and perpetuate asthma. With this in mind, it is clear that understanding the role of PAF-acether in the development of immediate into late asthma, and the unravelling of the precise cellular targets involved, should lead to the development of antagonists selective for each of the relevant targets and thus provide unique tools to test the different hypotheses concerning PAF-acether.

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