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Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet-activating factor: effect of anti-asthma drugs

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1 Exposure of guinea-pigs to aerosols of platelet activating factor (PAF) (0.01 to 100 $\mu g\,ml^{-1}$) induced a dose-dependent increased incidence of eosinophils in bronchoalveolar lavage fluid (BAL) at 48 h. Total leucocyte numbers and the percentages of lymphocytes and neutrophils were unchanged in BAL fluid.

2 Increased numbers of eosinophils were detected in BAL 1 h after exposure to PAF but eosinophilia was not maximal until 48 h. One week after exposure to PAF, the percentage of eosinophils in BAL was within the normal range.

3 Depletion of circulating platelets or neutrophils by intravenous injection of specific antisera did not modify accumulation of eosinophils in the airway lumen following inhalation of PAF (10 $\mu g\,ml^{-1}$).

4 PAF-induced pulmonary airway eosinophil accumulation was inhibited by treatment with SDZ 64–412, a selective PAF-antagonist, whether the compound was administered before, or 30 min after, inhalation of PAF.

5 Pulmonary airway eosinophil accumulation due to inhaled PAF (10 $\mu g\,ml^{-1}$) was inhibited by prior treatment with aminophylline, cromoglycate, ketotifen, dexamethasone and AH 21-132.

6 Pulmonary airway eosinophil accumulation due to inhaled PAF (10 $\mu g\,ml^{-1}$) was not inhibited by prior treatment with indomethacin, salbutamol or mepyramine.

Introduction

The presence of an eosinophilic infiltrate in the conducting airways of asthmatics was reported by Huber & Koessler in 1922. Eosinophil numbers in peripheral blood can be used as an index of disease severity in bronchial asthma (Horn *et al.*, 1975; Brown *et al.*, 1977) and the occurrence of eosinophils in bronchoalveolar lavage (BAL) fluid has been reported to precede late-onset responses to allergen in asthma patients (De Monchy *et al.*, 1985). However, only recently has the contribution of the eosinophil to the pathology of asthma been emphasised (Dahl & Venge, 1982; Frigas & Gleich, 1986). Toxic proteins derived from eosinophils have been demonstrated in sputum (Frigas *et al.*, 1981), BAL (De Monchy *et al.*, 1985) and blood (Dahl *et al.*, 1978) from asthma patients. Additionally, it is likely that lipid mediators such as peptidoleukotrienes (Verhagen *et al.*, 1984) and PAF (Lee *et al.*, 1984) may, in large part, be generated by eosinophils.

PAF has been reported to induce a selective accumulation of eosinophils in the airways of guinea-pigs (Aoki *et al.*, 1988a) and primates (Arnoux *et al.*, 1988). Hence, we have studied the dose relationship and time course of PAF-induced bronchial eosinophil accumulation in the guinea-pig and have examined the capacity of a range of anti-asthma drugs to inhibit this response.

Methods

Animals

Male Dunkin-Hartley guinea-pigs (400–600 g) were used for PAF exposure studies and antisera were raised in male New Zealand white rabbits (3–4 kg).

Antisera

Anti-serum to guinea-pig neutrophils Neutrophil-rich peritoneal exudates were induced by intraperitoneal injection of 5 ml of preformed immune complexes (bovine gamma globulin (BGG) + anti-BGG). After 4 h, guinea-pigs were killed (ether inhalation) and the peritoneal cavity was washed successively with four 10 ml aliquots of Hank's balanced salt solution (HBSS) containing EDTA (10 mM), HEPES (10 mM) and BSA (0.3%). Lavage fluid was centrifuged (200 *g* for 10 min) and the cell pellet resuspended in HBSS (1 ml). Approximately 3–5 × 10^7 cells were applied to a discontinuous Percoll gradient (densities: 1.07; 1.08; 1.09; 1.11) and centrifuged at 1000 *g* for 20 min at 20°C. At the interface between 1.09 and 1.11 density bands neutrophils were found in >95% purity. These cells were washed three times and resuspended in HBSS without EDTA or BSA. Neutrophils (2–3 × 10^7) in 1 ml were emulsified with an equal value of Freund's complete adjuvant (FCA) in a high speed blender and the emulsion was injected subcutaneously into the neck and flank of a rabbit. This procedure was repeated on two occasions at three-weekly intervals. Subsequently, blood was collected from anaesthetized (sodium pentothal 30–50 $mg\,kg^{-1}$, i.v.) rabbits by cardiac puncture and after clotting, was stored for 24 h. Serum was centrifuged (2000 *g* for 10 min), heat inactivated (56°C for 30 min) and adsorbed with guinea-pig red blood cells prior to storage as aliquots at −20°C.

Anti-serum to guinea-pig platelets Blood was collected by cardiac puncture of ether-anaesthetized guinea-pigs into sodium citrate (2.8% w/v). Blood was centrifuged (400 *g* for 10 min) at room temperature and platelet-rich plasma was centrifuged (1000 *g* for 10 min). The platelet pellet was resuspended in citrate buffer (1 ml) and approximately 5–10 × 10^8 platelets were emulsified with FCA. Rabbits were immunised according to the procedure described above.

Inhalation procedure

Guinea-pigs were restrained by the neck, with the snout inserted into a nose-cone which was connected to the outlet of

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Table 1 Time course of eosinophil accumulation in pulmonary airways of guinea-pigs exposed to an aerosol of PAF ($10\,\mu g\,ml^{-1}$)

Time	n	Total cells ($\times 10^6$)	% Cell populations in BAL			
			Mac	Eos	Lym	Neut
0	15	17.5 ± 2.5	83 ± 1	8 ± 1	7 ± 1	2.1 ± 0.4
1 h	10	19.9 ± 1.4	82 ± 2	11 ± 1†	5 ± 2	1.0 ± 0.1
4 h	10	21.0 ± 2.0	79 ± 2	14 ± 2*	5 ± 1	2.0 ± 0.1
24 h	10	17.9 ± 1.5	74 ± 3*	16 ± 2*	6 ± 1	3.0 ± 0.1
48 h	20	19.9 ± 2.5	70 ± 2*	21 ± 2*	5 ± 1	2.0 ± 0.1
72 h	10	19.6 ± 2.0	80 ± 2	13 ± 2†	6 ± 1	1.4 ± 0.6
1 week	10	18.3 ± 1.6	82 ± 8	9 ± 1	2 ± 1	1.0 ± 0.2
2 week	4	23.3 ± 2.9	85 ± 2	6 ± 1	7 ± 1	1.3 ± 0.5

Results are expressed as mean ± s.e.mean. Student's t test for groups of unequal size was used to assess the statistical significance of observed differences between no exposure (time 0) and various time points after exposure to PAF; †$P < 0.05$, *$P < 0.005$. Mac = macrophages; Eos = eosinophils; Lym = lymphocytes; Neut = neutrophils; BAL = bronchoalveolar lavage fluid.

a Devilbiss nebuliser. Aerosols were generated by use of compressed air (7 litres min^{-1}). Guinea-pigs were exposed for 1 h to aerosols generated from 10 ml of a solution of PAF (0.01 to $100\,\mu g\,ml^{-1}$) in BSA (0.25%) or BSA (0.25%) alone. For kinetic studies, animals were exposed to PAF ($10\,\mu g\,ml^{-1}$) for 1 h and lungs were washed 1, 4, 24, 48, 72 h and 1 or 2 weeks after exposure. For dose-effect studies, guinea-pigs were exposed to aerosols of PAF at increasing concentrations (0–$100\,\mu g\,ml^{-1}$) and eosinophilia in BAL was determined 48 h after exposure.

Bronchoalveolar lavage

Guinea-pigs were killed with sodium pentobarbitone ($100\,mg\,kg^{-1}$, i.p.), the trachea was cannulated to allow the lungs to be washed with six aliquots of modified HBSS (10 ml). Total fluid recovery exceeded 85%. Cell suspensions were concentrated by low speed centrifugation (200 g for 10 min) and the cell pellet resuspended in modified HBSS (1 ml). Total cell counts were made in a haemocytometer. Differential cell counts were made from smears fixed in methanol (1 min) and stained with Leishman's stain (4 min). A total of 500 cells per smear were counted by light microscopy under oil immersion ($\times$ 1000), in order to differentiate cell types.

Drugs

PAF (C_{16}; Novobiochem) was dissolved in ethanol at $1\,mg\,ml^{-1}$ and stored at −18°C. Dilutions of PAF were made in 0.25% bovine serum albumin (BSA, Fluka) in physiological saline.

Ketotifen, aminophylline, cromoglycate, mepyramine, SDZ 64-412, 2,3-dihydro-5-[4-(3,4,5-trimethoxyphenylethyl)phenyl]-imidazo[2,1-α]isoquinolinium hydrochloride, AH 21-132, *cis*-6-(*p*-acetoamidophenyl)-1,2,3,4a,10b-hexahydro-8,9-demethoxy-2-methylbenzo-[c][1,6]-naphthyridine, (Sandoz), salbutamol (Glaxo) and indomethacin (Sigma) were dissolved in physiological saline; dexamethasone (Sigma) was dissolved in a mixture of ethanol and polyethylene glycol (50:50 v/v).

Drug administration

With the exception of SDZ 64-412, drug solutions were loaded into osmotic minipumps (Alzet, 2001 with a delivery rate of $1\,\mu l\,h^{-1}$) and implanted subcutaneously in the nuchal region of guinea-pigs during ether anaesthesia. Five days after implantation of minipumps, animals were exposed to an aerosol of PAF ($10\,\mu g\,ml^{-1}$) and 48 h later, BAL was collected. SDZ 64-412, a selective PAF-receptor antagonist, was given orally either 1 h before PAF challenge or 30 min after Paf challenge at a dose of $10\,mg\,kg^{-1}$.

Statistical comparisons

Student's t test (two tailed) was used for comparison between treatments.

Results

Time-course of eosinophil accumulation

In untreated animals, BAL fluid yielded 17.5 ± 2.5 × 10^6 leukocytes (n = 15). These comprised 83 ± 1% macrophages, 8 ± 1% eosinophils, 7 ± 1% lymphocytes and 2 ± 0.4% neutrophils. Occasionally, other cell types such as ciliated and non-ciliated epithelial cells were observed; however, since these cells typically formed strips or clumps, they could not be quantified. In animals exposed to PAF ($10\,\mu g\,ml^{-1}$), the leucocyte content of BAL was examined at 1, 4, 24, 48, 72 h, as well as 1 and 2 weeks after exposure. The percentage of eosinophils in BAL 1 h after cessation of aerosol exposure was significantly greater than that in untreated animals ($P < 0.05$) but maximal eosinophilia was not observed until 48 h had elapsed (Table 1, Figure 1). Eosinophil numbers in PAF-treated animals remained significantly greater than in untreated animals at 72 h ($P < 0.05$), but had returned to values within

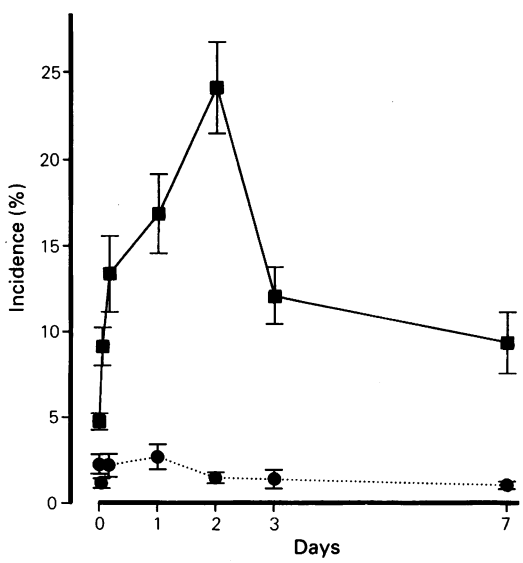

Figure 1 Incidence of eosinophils (■) and neutrophils (●) in bronchoalveolar lavage fluid collected from guinea-pigs at various times after inhalation of an aerosol of PAF ($10\,\mu g\,ml^{-1}$) for 1 h. Bars depict s.e.mean and n = 10–20 animals per time point.

Table 2 The effect of exposure of guinea-pigs to increasing concentrations of aerosolised PAF on BAL cells recovered 48 h after exposure

	n	*Total cell* ($\times 10^6$)	*% Cell populations in BAL* Mac	Eos	Lym	Neut
No treatment	15	17.5 ± 2.5	83 ± 1	8 ± 1	7 ± 1	2.1 ± 0.4
BSA 0.25%	15	25.1 ± 3.2	80 ± 2	10 ± 2	7 ± 1	2.0 ± 0.5
PAF 0.01	10	20.4 ± 1.9	76 ± 2	12 ± 1	8 ± 1	2.3 ± 0.6
($\mu g ml^{-1}$) 0.1	10	19.3 ± 2.6	72 ± 2*	15 ± 2†	7 ± 1	2.0 ± 0.7
1.0	10	20.8 ± 1.5	71 ± 2*	19 ± 2*	6 ± 1	1.8 ± 0.5
10.0	10	18.9 ± 1.6	69 ± 2*	23 ± 2*	6 ± 1	1.4 ± 0.3
100.0	10	23.3 ± 2.9	62 ± 3*	28 ± 4*	7 ± 1	1.3 ± 0.3

Results are expressed as mean ± s.e.mean. Student's *t* test for groups of unequal size was used to assess the statistical significance of observed differences between bovine serum albumin (BSA) and PAF-treated groups; † $P < 0.05$; * $P < 0.005$. Mac = macrophages; Eos = eosinophils; Lym = lymphocytes; Neut = neutrophils. BAL = bronchoalveolar lavage fluid.

the normal range after 1 week (Table 1). No significant changes in leucocyte number were observed, other than a decrease in the percentage of macrophages 24 and 48 h after exposure to PAF (Table 1). Exposure of guinea-pigs to an aerosol of BSA did not significantly increase the percentage of eosinophils in BAL fluid at 24 (8.7 ± 1.4%; $n = 7$) or 48 h (10 ± 2%; $n = 15$).

Dose-effect relationship

Since maximal eosinophil accumulation was observed 48 h after exposure to an aerosol of PAF ($10 \mu g ml^{-1}$), this time-point was chosen to examine the effect on leucocyte populations in BAL of aerosols of PAF at concentrations between 0.01 and $100 \mu g ml^{-1}$. Increasing concentrations of Paf induced a dose-related increase in the percentage of eosinophils observed in BAL, with significant eosinophilia being detectable after inhalation of an aerosol containing $0.1 \mu g ml^{-1}$ of PAF (Table 2). There was a concomitant decrease in the percentage of macrophages recovered in BAL, whilst neutorphil or lymphocyte populations remained unaltered (Table 2).

Effect of neutrophil or platelet depletion

To ascertain whether circulating platelets or neutrophils participate in eosinophil recruitment to the lungs after exposure to an aerosol of PAF ($10 \mu g ml^{-1}$), each cell type was depleted selectively by specific antisera.

In vitro specificity of the anti-neutrophil serum (ANS) used for this study has been reported elsewhere (Hutson *et al.*, 1989). Intravenous injection of ANS ($2 ml kg^{-1}$) produced a neutropaenia in blood which was maximal (>96%) at 24 h and persisted for 48 h with recovery being evident at 96 h (control 4490 ± 600 μl^{-1}; ANS 24 h, 260 ± 68 μl^{-1}; ANS 48 h, 993 ± 227 μl^{-1}; ANS 96 h, 3836 ± 558 μl^{-1}; $n = 3$). A marginal reduction of platelet counts (at 24 h) was not significant.

Intravenous injection of anti-platelet serum (APS) caused depletion of 94.5 ± 1.7% of circulating platelets 24 h after injection (control 448 ± 63.1 × $10^3 \mu l^{-1}$; APS 24 h, 23.2 ± 1.9 × $10^3 \mu l^{-1}$; $n = 5$). Recovery from platelet depletion was more rapid than from neutrophil depletion, so that platelet counts were within the normal range by 48 h.

Antisera were injected intravenously 24 h prior to PAF exposure, to induce maximal depletion of each cell type. Neither platelet depletion nor neutrophil depletion affected the basal cell numbers recovered in BAL or the pulmonary airway eosinophil accumulation that occurred 48 h after exposure to an aerosol of PAF ($10 \mu g ml^{-1}$) (Figure 2).

Effect of a PAF-receptor antagonist

Eosinophil accumulation in the pulmonary airways was fully inhibited by administration of a selective PAF-receptor

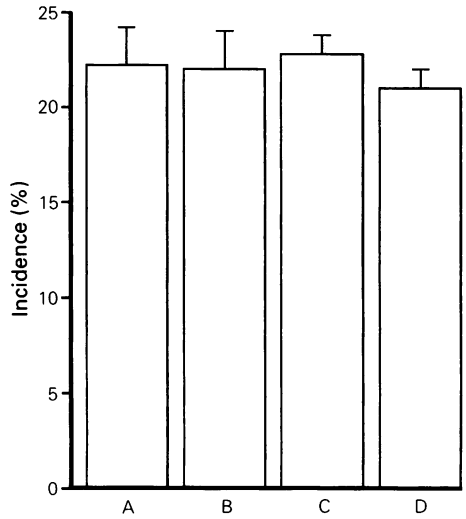

Figure 2 Incidence of eosinophils in bronchoalveolar lavage fluid collected from guinea-pigs 48 h after inhalation of an aerosol of PAF ($10 \mu g ml^{-1}$) following no treatment (A), pretreatment with normal rabbit serum (B), anti-platelet antiserum (C) or anti-neutrophil antiserum (D). Bars depict s.e.mean and $n = 10$ animals per treatment.

Table 3 The effect of anti-asthma drugs on PAF-induced eosinophil accumulation in pulmonary airways of the guinea-pig

Treatment	*Dose* ($mg kg^{-1}$)	*Total cells* ($\times 10^6$)	*Eosinophils* (%)	P^1
Saline	—	20.0 ± 2.1	22.2 ± 1.6	
SDZ 64-412	10.0	24.7 ± 4.4	10.0 ± 2.5	<0.001
Cromoglycate	1.0	17.8 ± 2.3	13.4 ± 2.0	<0.01
Ketotifen	1.0	16.9 ± 0.9	11.1 ± 1.1	<0.001
	0.1	18.0 ± 2.0	14.0 ± 0.9	<0.001
AH 21-132	1.0	18.7 ± 0.8	9.8 ± 0.8	<0.001
	0.1	15.6 ± 1.0	17.7 ± 1.4	<0.05
Indomethacin	1.0	14.9 ± 1.1	18.8 ± 1.2	NS
Salbutamol	1.0	12.8 ± 0.9	19.2 ± 2.0	NS
Mepyramine	1.0	24.7 ± 3.5	20.4 ± 3.1	NS
Dexamethasone	1.0	18.3 ± 2.4	8.8 ± 1.0	<0.001
Aminophylline	10.0	20.6 ± 1.6	10.3 ± 1.6	<0.001

Results are expressed as mean ± s.e.mean. [1] Student's *t* test was used to assess the statistical significance of observed differences between drug treatment and saline treatment of the eosinophil response. Drugs were given subcutaneously by osmotic minipumps implanted for 7 days, except SDZ 64-412 which was given orally 1 h before aerosol exposure. Each treatment group comprises 10 animals exposed to PAF $10 \mu g ml^{-1}$.

antagonist SDZ 64-412 (Handley et al., 1988) (10 mg kg^{-1}, p.o.), 1 h before exposure to an aerosol of PAF (10 μg ml^{-1}) (Table 3). In a separate study, SDZ 64-412 (10 mg kg^{-1}, p.o.) was administered 30 min after exposure to an aerosol of PAF (10 μg ml^{-1}). Guinea-pigs which were not exposed to PAF had basal eosinophil numbers in BAL of 5.1 $\pm$ 0.9% in the absence of drug treatment; sham-treated guinea-pigs exposed to an aerosol of PAF had a pulmonary airway eosinophilia of 14.9 $\pm$ 1.6% ($P < 0.0005$ compared to no treatment) and animals exposed to PAF and subsequently treated with SDZ 64-412 exhibited a pulmonary airway eosinophilia of 6.5 $\pm$ 3.2%, which was not significantly different from the incidence of eosinophils in untreated animals.

Effect of pretreatment with anti-asthma drugs

Daily pretreatment of guinea-pigs with aminophylline (10 mg kg^{-1}), cromoglycate (1 mg kg^{-1}), ketotifen (0.1–1 mg kg^{-1}), AH 21-132 (0.1–1 mg kg^{-1}) or dexamethasone (1 mg kg^{-1}) caused a significant decrease in the percentage of eosinophils in BAL, 48 h after exposure to PAF (10 μg ml^{-1}), whereas pretreatment with mepyramine (1 mg kg^{-1} day^{-1}), indomethacin (1 mg kg^{-1} day^{-1}) or salbutamol (1 mg kg^{-1} day^{-1}) did not diminish eosinophil accumulation significantly (Table 3). The lack of inhibition observed with the latter three drugs was not related to the administered dose since arachidonic acid-induced bronchoconstriction was reduced (although not fully inhibited) in indomethacin-treated animals and histamine-induced bronchoconstriction was fully inhibited in mepyramine-treated guinea-pigs (data not shown). The effectiveness of salbutamol was assessed by β-adrenoceptor desensitization which was observed after 5 days of treatment.

Discussion

Platelet activating factor (PAF), which was first identified in the supernatant of rabbit basophils challenged with antigen (Benveniste et al., 1972), is now recognised as a potent inflammatory mediator with a wide range of biological activities (Braquet et al., 1987). PAF and lyso-PAF, the precursor and metabolite of PAF, are released into the airway lumen of lungs from sensitized guinea-pigs after exposure to antigen (Fitzgerald et al., 1986) and increased levels of lyso-PAF have been detected in serum of asthma patients in whom there was a late-onset reaction to allergen (Nakamura et al., 1987). PAF is released during allergic reactions, is chemoattractant for both neutrophils and eosinophils *in vitro* (Wardlaw et al., 1986) and induces accumulation of such cells *in vivo* (Dewar et al., 1984; Henocq & Vargaftig, 1986); hence, it is reasonable to anticipate that PAF formation will contribute to the accumulation of such inflammatory cells in the lungs of asthma patients.

In the present experiments, exposure of guinea-pigs to an aerosol of PAF induced a selective, dose-dependent accumulation of eosinophils in the airway lumen which was maximal at 48 h. Microscopically, these eosinophils appeared larger than eosinophils from untreated guinea-pigs, yet there was little evidence of degranulation. An attempt was made to separate eosinophils on the basis of their density by use of discontinuous Percoll gradients (densities 1.07, 1.08, 1.09, 1.10). Eosinophils sedimented at the interface of layers 1.08/1.09 (hypodense) and 1.09/1.10 (normodense) in normal and PAF-treated guinea-pigs. Variability of the ratio between hypodense and normodense eosinophils was too large to allow an assessment of changes in density.

Guinea-pigs were exposed to PAF over a prolonged period (1 h) in order to avoid the severe bronchospasm that can occur upon exposure to an aerosol of PAF (Lefort et al., 1984) and which would limit entry of PAF into the airways. Guinea-pigs are obligatory nose-breathing animals so that only small quantities of aerosols enter the pulmonary airways. This has been shown in studies with radiolabelled BSA (Broder et al., 1978) or ovalbumin (Richerson, 1972) which estimated that between 0.01 and 0.05% of the total dose is deposited within guinea-pig airways and use of radiolabelled materials for imaging has confirmed this conclusion (Gazeley et al., 1986). It may be presumed that nanogram quantities of PAF enter the lung in the present experiments and that the major proportion of inhaled PAF will have been deposited in the nasal mucosa.

Despite having a short half-life *in vivo* (Latrigue-Mattei et al., 1984), PAF can cause chronic inflammatory changes when injected intradermally (Dewar et al., 1984) or instilled into the lungs of experimental animals (Camussi et al., 1983). Results of the present study confirm that the cellular reaction to PAF is protracted, since maximal eosinophilia was observed 48 h after a single exposure to an aerosol of PAF. In the guinea-pig mesentery, local application of PAF induces persisting thrombosis that can be accounted for by a protracted formation of PAF by vascular endothelium (Bourgain et al., 1986). An analogous mechanism may contribute to eosinophil accumulation, since the accumulation of eosinophils within the airways was diminished by SDZ 64-412, even when administered 30 min after exposure to PAF. This observation suggests that either PAF is not destroyed rapidly on the epithelial surface, possibly by binding to glycoproteins in the mucus layer, or that PAF induces sustained generation of PAF. Both alveolar macrophages and eosinophils are capable of generating PAF when activated by a variety of stimuli (Arnoux et al., 1980; 1982; Lee et al., 1984). These cells may be appropriate candidates for sustained PAF generation.

It has been proposed that during eosinophil accumulation in the guinea-pig, platelet activation is an obligatory intermediate step (Lellouch-Tubiana et al., 1988). It is known that intravenous injection of PAF causes entrapment of platelets and neutrophils (Dewar et al., 1984) to be succeeded by emigration of eosinophils (McManus, 1987). Nonetheless, the present experiments show that platelet depletion with lytic anti-serum did not diminish the accumulation of eosinophils in guinea-pig airways exposed to an aerosol of PAF. In the study of Lelluch-Tubiana et al. (1988), PAF was given intravenously, which may account for the differing observations since there are clear differences between the effects upon the guinea-pig lung of PAF given by the intravenous and inhaled routes. Intravenous PAF causes a platelet-dependent bronchoconstriction (Vargaftig et al., 1980) and airway hyperreactivity (Mazzoni et al., 1985), whereas bronchoconstriction due to an aerosol of PAF is not dependent upon circulating platelets (Lefort et al., 1984). In the present experiments, an absence of circulating neutrophils did not influence eosinophil accumulation after inhalation of PAF, in agreement with recent findings that neutrophil depletion did not affect the magnitude of late-onset airway obstruction or eosinophil accumulation in sensitized guinea-pigs challenged with an aerosol of antigen (Aoki et al., 1988b; Hutson et al., 1989). It is of interest to note that at no time after PAF inhalation was an increased neutrophil infiltration observed in BAL fluid. This observation is in marked contrast to the pre-eminent accumulation of neutrophils which is evident after intradermal injection of PAF in guinea-pigs (Dewar et al., 1984) and man (Archer et al., 1985). It is possible that airway and skin vasculature differ in their response to PAF.

Results of the present study reveal that pretreatment of guinea-pigs with ketotifen, aminophylline, cromoglycate, dexamethasone or AH 21-132 inhibited pulmonary eosinophil accumulation that followed inhalation of an aerosol of PAF. On the other hand, mepyramine, indomethacin or salbutmol were without effect. Cyclo-oxygenase inhibitors do not inhibit eosinophil accumulation (Spicer et al., 1985; Fugner, 1989) and anti-histamines are not considered to affect eosinophil accumulation, since most experiments using antigen as the stimulus are conducted under cover of an antagonist of histamine to prevent death from anaphylaxis. The action of β-

adrenoceptor agonists is more controversial with one study reporting an inhibition of antigen-induced eosinophilia by fenoterol (Fugner, 1989) and another confirming our finding of no inhibition with albuterol (Hutson *et al.*, 1988). Whether these differences represent different dose effects, differences in duration of action or other actions of the drugs is not clear at present.

Our observations are consistent with the available clinical observations that a reduction of eosinophils in blood, sputum or lungs occurs in atopic asthmatic patients following treatment with cromoglycate (Diaz *et al.*, 1984) or ketotifen (Gobel, 1978) and in patients with intrinsic bronchial asthma when treated with glucocorticosteroids (Horn *et al.*, 1975). Furthermore, theophylline and glucocorticosteroids, but not cromoglycate or salbutamol, inhibit the production of an eosinophil-activating factor by peripheral blood monocytes obtained from individuals with moderate eosinophilia (Thorne *et al.*, 1988). The question as to whether these drugs share a common site of action or whether they act at different points along the pathway from eosinophilpoiesis to accumulation and degranulation of eosinophils in the bronchial walls and mucus of asthmatics remains to be determined.

References

AOKI, S., BOUBEKEUR, K., BURROWS, L., MORLEY, J. & SANJAR, S. (1988a). Recruitment of eosinophils by platelet activating factor (PAF) in the guinea-pig lung. *J. Physiol.*, **394**, 130P.

AOKI, S., BOUBEKEUR, K., KRISTERSSON, A., MORLEY, J. & SANJAR, S. (1988b). Is allergic airway hyperreactivity of the guinea-pig dependent on eosinophil accumulation in the lung? *Br. J. Pharmacol.*, **94**, 365P.

ARCHER, C.B., PAGE, C.P., MORLEY, J. & MACDONALD, D.M. (1985). Accumulation of inflammatory cells in response to intracutaneous platelet activating factor (Paf-acether) in man. *Br. J. Dermatol.*, **112**, 285–290.

ARNOUX, B., DUVAL, D. & BENVENISTE, J. (1980). Release of platelet-activating factor (PAF-acether) from alveolar macrophages by the calcium ionophore A 23187 and phagocytosis. *Eur. J. Clin. Invest.*, **10**, 437–441.

ARNOUX, B., SIMOES-CAEIRO, M.H., LANDES, A., MATHIEU, M., DUROUX, P. & BENVENISTE, J. (1982). Alveolar macrophages from asthmatic patients release platelet-activating factor (Paf-acether) and lyso Paf-acether when stimulated with the specific allergen. *Am. Rev. Respir. Dis.*, **125**, A50.

ARNOUX, B., DENJEAN, A., PAGE, C.P., NOLIBE, D., MORLEY, J. & BENVENISTE, J. (1988). Accumulation of platelets and eosinophils in baboon lung following PAF-acether challenge: inhibition by Ketotifen. *Am. Rev. Respir. Dis.*, **137**, 855–860.

BENVENISTE, J., HENSON, P.M. & COCHRANE, C.G. (1972). Leucocyte dependent histamine release from rabbit platelets: The role of IgE, basophil and a platelet activating factor. *J. Exp. Med.*, **136**, 1356–1377.

BOURGAIN, R.H., MAES, L., ANDRIES, R. & BRAQUET, P. (1986). Thrombus induction by endogenic paf-acether and its inhibition by ginko biloba extracts in the guinea-pig. *Prostaglandins*, **32**, 142–144.

BRAQUET, P., TOUQUI, L., SHEN, T.Y. & VARGAFTIG, B.B. (1987). Perspectives in platelet-activating factor research. *Pharmacol. Rev.*, **39**, 97–145.

BRODER, I., ROGERS, S., CHAMBERLAIN, D.W. & MILNE, E.N.C. (1978). Model of allergic bronchoconstriction in the guinea-pig. 1. Characteristics of the system. *Clin. Immunol. Immunopathol.*, **9**, 1–15.

BROWN, H.M., STOREY, G. & JACKSON, F.A. (1977). Beclomethasone Dipropionate aerosol in long-term treatment of perennial and seasonal asthma in children and adults: A report of five-and-half years' experience in 100 asthmatic patients. *Br. J. Clin. Pharmacol.*, **4**, 259S–267S.

CAMUSSI, G., PAWLOWSKI, I., TETTA, C., ROFFINELLO, C., ALBERTON, M., BRENTJENS, J. & ANDRES, G. (1983). Acute lung inflammation induced in the rabbit by local instillation of 1-0-octadecyl-2-acetyl-syn-glyceryl-3-phosphorylcholine or of a native platelet-activating factor. *Am. J. Pathol.*, **115**, 78–88.

DAHL, R. & VENGE, P. (1982). Role of the eosinophil in bronchial asthma. *Eur. J. Respir. Dis.*, **63**, 23–28.

DAHL, R., VENGE, P. & OLSSON, I. (1978). Variations of blood eosinophils and eosinophil cationic protein in serum in patients with bronchial asthma. *Allergy*, **33**, 211–215.

DE MONCHY, J.G.R., KAUFFMAN, H.F., VENGE, P., KOETER, G.H., JANSEN, H.M., SLUTTER, H.J. & DE VRIES, K. (1985). Broncho alveolar eosinophilia during allergen-induced late asthmatic reactions. *Am. Rev. Respir. Dis.*, **131**, 373–377.

DEWAR, A., ARCHER, C.B., PAUL, W., PAGE, C.P., MACDONALD, D.M. & MORLEY, J. (1984). Cutaneous and pulmonary histopathological responses to platelet activating factor (Paf-acether) in the guinea-pig. *J. Pathol.*, **144**, 24–34.

DIAZ, P., GALLEGUILLOS, F.R., GONZALES, M.C., PANTIN, F.C.A. & KAY, A.B. (1984). Bronchoalveolar lavage in asthma: the effect of disodium cromoglycate (cromolyn) on leukocyte counts, immunoglobulins, and complement. *J. Allergy Clin. Immunol.*, **74**, 41–48.

FITZGERALD, M.F., MONCADA, S. & PARENTE, L. (1986). The anaphylatic release of platelet-activating factor from perfused guinea-pig lungs. *Br. J. Pharmacol.*, **88**, 149–153.

FRIGAS, E. & GLEICH, G.J. (1986). The eosinophil and the pathophysiology of asthma. *J. Allergy Clin. Immunol.*, **77**, 527–537.

FRIGAS, E., LOEGERING, D.A., SOLLEY, G.O., FARROW, G.M. & GLEICH, G.J. (1981). Elevated levels of eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin. Proc.*, **56**, 345–353.

FUGNER, A. (1989). Formation of oedema and accumulation of eosinophils in the guinea pig lung. Inhibition by inhaled beta-stimulants. *Int. Arch. Allergy Clin. Immunol.*, **88**, 225–227.

GAZELEY, M.J.S., LEES, I.W., PAYNE, A.N., WEBBON, P.M. & WOOLLEY, G.E. (1986). Anatomical deposition and early distribution of an inhaled radioaerosol in conscious and anaesthetised rats. *Br. J. Pharmacol.*, **87**, 122P.

GOBEL, P. (1978). The protective effect of ketotifen in bronchial asthma. *J. Int. Med. Res.*, **6**, 79–85.

HANDLEY, D.A., VAN VALEN, R.G., MELDEN, M.K., HOULIHAN, W.J. & SAUNDERS, R.N. (1988). Biological effects of the orally active platelet activating factor receptor antagonist SDZ 64-412. *J. Pharmacol. Exp. Ther.*, **247**, 617–622.

HENOCQ, E. & VARGAFTIG, B.B. (1986). Accumulation of eosinophils in response to intracutaneous paf-acether and allergens in man. *Lancet*, **i**, 1378–1379.

HORN, B.R., ROBIN, E.D., THEODORE, J. & VAN KESSEL, A. (1975). Total eosinophil counts in the management of bronchial asthma. *New. Engl. J. Med.*, **292**, 1152–1155.

HUBER, H.L. & KOESSLER, K.K. (1922). Pathology of asthma. *Arch. Intern. Med.*, **30**, 689–760.

HUTSON, P.A., HOLGATE, S.T. & CHURCH, M.K. (1988). The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of nonanesthetized guinea pigs. *Am. Rev. Respir. Dis.*, **138**, 1157–1163.

HUTSON, P.A., SANJAR, S., KINGS, M., HOLGATE, S.T. & CHURCH, M.K. (1989). Evidence that neutrophils do not participate in the late phase airways response provoked by ovalbumin inhalation in conscious sensitised guinea-pigs. *Am. Rev. Respir. Dis.*, (in press).

LATRIGUE-MATTEI, C., GODENECHE, D., CHABARD, J.L., PETIT, J. & BERGER, J.A. (1984). Pharmacokinetic study of 3H-labelled PAF-acether II. Comparison with 3H-labelled lyso-PAF-acether after intravenous administration in the rabbit and protein binding. *Agents Actions*, **15**, 643–648.

LEE, T.C., LENIHAN, D.J., MALONE, B., RODDY, L.L. & WASSERMAN, S.I. (1984). Increased biosynthesis of platelet activating factor in activated human eisinophils. *J. Biol. Chem.*, **259**, 5526–5530.

LEFORT, J., ROTILIO, D. & VARGAFTIG, B.B. (1984). The platelet-independent release of thromboxane A2 by Paf-acether from guinea-pig lungs involves mechanisms distinct from those for leukotriene. *Br. J. Pharmacol.*, **82**, 565–575.

LELLOUCH-TUBIANA, A., LEFORT, J., SIMON, M-T., PFISTER, A. & VARGAFTIG, B.B. (1988). Eosinophil recruitment into guinea pig lungs after PAF-acether and allergen administration. *Am. Rev. Respir. Dis.*, **137**, 948–954.

MAZZONI, L., MORLEY, J., PAGE, C.P. & SANJAR, S. (1985). Induction of hyperreactivity by platelet activating factor in the guinea-pig. *J. Physiol.*, **365**, 107P.

McMANUS, L.M. (1987). Acute lung injury induced by intravascular platelet activating factor. In *New Horizons in Platelet Activating Research*. ed. Winslow, C.M. & Lee, M.L. pp 233–244. Chichester, New York: John Wiley and Sons Ltd.

NAKAMURA, T., MORITA, Y., KURIYAMA, M., ISHIHARA, K., ITO, K. & MIYAMOTO, T. (1987). Platelet-activating factor in late asthmatic response. *Int. Arch. Allergy Appl. Immunol.*, **82**, 57–61.

RICHERSON, H.B. (1972). Acute experimental hypersensitivity pneumonitis in the guinea-pig. *J. Lab. Clin. Med.*, **79**, 745–757.

SPICER, B.A., LAYCOCK, S.M. & SMITH, H. (1985). The effects of drugs on leucocyte changes following the injection of antigen into the peritoneal cavities of actively sensitised rats. *Agents Actions*, **17**, 498–505.

THORNE, K.J.I., RICHARDSON, B.A., BUTTERWORTH, A.E., HAY, I. & HIGENBOTTAM, T.W. (1988). Effect of drugs used in the treatment of asthma on the production of eosinophil-activating factor by monocytes. *Int. Arch. Allergy Appl. Immunol.*, **85**, 257–259.

VARGAFTIG, B.B., LEFORT, J., CHIGNARD, M. & BENVENISTE, J. (1980). Platelet-activating factor induces a platelet dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. *Eur. J. Pharmacol.*, **65**, 185–192.

VERHAGEN, J., BRUYNZEEL, P.L.B., KOEDAM, J.A., WASSINK, G.A., DE BOER, M., TERPSTRA, G.K., KREUKNIET, J., VELDINK, G.A. & VLIEGENTHART, J.F.G. (1984). Specific leukotriene formation by purified human eosinophils and neutrophils. *FEBS Lett.*, **168**, 23–28.

WARDLAW, A.J., MOQBEL, R., CROMWELL, O. & KAY, A.B. (1986). Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J. Clin. Invest.*, **78**, 1701–1706.

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