Effect of a 5-lipoxygenase inhibitor and leukotriene antagonist (PF 5901) on PAF-induced airway responses in neonatally immunized rabbits

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1 Aerosol administration of platelet activating factor (PAF) ($80 \mu\text{g ml}^{-1}$ for 60 min) to neonatally immunized rabbits caused bronchoconstriction which was far in excess of that produced by a comparable aerosol of bovine serum albumin (BSA), the carrier molecule for PAF. Bronchoconstriction of a similar magnitude was elicited by PAF in immunized, sham-immunized and normal rabbits.

2 Aerosol administration of PAF to immunized rabbits induced enhanced airway responsiveness to inhaled histamine in all animals tested, 24 h and 72 h after exposure. In not all cases had airways responsiveness returned to basal levels at 1 week following PAF challenge. In contrast, following exposure of immunized rabbits to BSA, no significant changes in airway responsiveness to histamine were evident at any of the measured time points.

3 A significant increase in the total number of inflammatory cells recovered in bronchoalveolar lavage (BAL) fluid was determined 24 h and 72 h following PAF exposure in immunized rabbits. This was associated with a significant increase in the number of neutrophils and eosinophils. Similar changes were observed following exposure of PAF to normal and sham-immunized rabbits. No change in the total number of inflammatory cells was obtained in BAL after BSA challenge to immunized rabbits; however, neutrophil numbers were significantly increased.

4 PF 5901, a specific inhibitor of the 5-lipoxygenase pathway of arachidonic acid metabolism and a leukotriene D_4 antagonist, at a dose of 10 mg (direct intratracheal administration) significantly inhibited the airway resistance (R_L) component of the bronchoconstriction induced by PAF in neonatally-immunized rabbits. Doses of 10 mg, 3 mg and 1 mg PF 5901 (direct intratracheal administration) were sufficient to inhibit significantly the PAF-induced increase in airways responsiveness to inhaled histamine in immunized rabbits. PF 5901 however, failed to alter the pulmonary cell infiltration induced by PAF, as assessed by BAL.

5 We suggest from the results of the present study that PAF induces consistent and long-lasting increases in airways responsiveness to histamine in immunized rabbits, which is mediated, at least in part, by products of the 5-lipoxygenase metabolic pathway. Furthermore, the inability of PF5901 to inhibit the influx of inflammatory cells into the airway lumen following PAF challenge may suggest that bronchial hyperresponsiveness and cellular infiltration are not strictly associated events.

Keywords: PAF; leukotrienes; rabbit airways; inflammation; airway hyperresponsiveness

Introduction

Bronchial asthma is characterized by bronchoconstriction, mucosal oedema, mucus hypersecretion, bronchial hyperresponsiveness and a recruitment of leucocytes, in particular eosinophils, into the airways. It has been suggested that platelet activating factor (PAF) is a mediator of this disease as it can reproduce many of these features, both in experimental animals and in man (Page, 1988). Consequently, much research has been focussed on the elucidation of the precise role of this family of phospholipids in allergy and inflammation.

PAF has been shown to induce both acute bronchoconstriction and a longer-lasting bronchial hyperresponsiveness in a variety of laboratory animals (reviewed in Page, 1988). However, the response to PAF in man is variable (Cuss *et al.*, 1986; Rubin *et al.*, 1987; Chung & Barnes, 1989; Chung *et al.*, 1989; Kaye & Smith, 1990; Lai *et al.*, 1990; Spencer *et al.*, 1990b), the basis of which is not known. Similarly, the response of normal rabbits to PAF has also been shown to be variable, where bronchial hyperresponsiveness was induced in some, but not all normal animals studied (Coyle *et al.*, 1990).

The mechanisms by which PAF produces bronchoconstriction and airway hyperresponsiveness has yet to be determined. PAF has been shown to enhance the release of leukotrienes from isolated perfused lung of the rabbit (Voelkel *et al.*, 1982) and from chopped rat lung (Beaubien *et al.*, 1984). A role for the lipoxygenase products of arachidonic acid, particularly leukotrienes formed by the 5-lipoxygenase pathway, has been suggested to account for a number of PAF-induced effects including bronchoconstriction (Bonnet *et al.*, 1983), vascular permeability, and in the acute increase in airway responsiveness to histamine following PAF exposure (Anderson & Fennessy, 1988). Similar findings have been obtained *in vitro*, where hyperresponsiveness to PAF in lungs from actively sensitized guinea-pigs is partly related to the generation of lipoxygenase products (Pretolani *et al.*, 1989).

The breakdown of arachidonic acid leads to the formation of the sulphidopeptide leukotrienes C_4 (LTC_4), D_4 (LTD_4) and E_4 (LTE_4) which induce bronchoconstriction (Drazen *et al.*, 1980), airway hyperresponsiveness (O'Hickey *et al.*, 1991), mucus secretion (Marom *et al.*, 1982) and increased vascular permeability (Hua *et al.*, 1985), and leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (5-HETE). LTB_4 and 5-HETE are potent stimulators of leukocyte functions, including the chemotaxis, chemokinesis and aggregation of

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polymorphonuclear leukocytes (Ford-Hutchinson *et al.*, 1981; Ford-Hutchinson, 1991).

In the present study, the ability of a specific and selective leukotriene synthesis inhibitor and LTD₄ antagonist, PF 5901 (2-[3-(1-hydroxyhexyl)phenoxyethyl]quinoline hydrochloride) (Van inwegen *et al.*, 1987; Evans *et al.*, 1991) to influence bronchoconstriction, pulmonary cell infiltration and airway hyperresponsiveness induced by PAF in spontaneously breathing rabbits was investigated. Preliminary findings of this work have been presented to the American Thoracic Society (Herd & Page, 1991) and to the British Pharmacological Society (Herd *et al.*, 1991).

Methods

Animals

New Zealand White (NZW) rabbits (Froxfield Farms, Petersfield, Hants) of either sex were used throughout the study. Rabbits used were allergen-immunized, sham-immunized or normal. The immunization of neonatal rabbits was performed by a previously described method (McKenniff *et al.*, 1991). Briefly, rabbits were injected intraperitoneally (0.5 ml) within 24 h of birth with *Alternaria tenuis* extract (1 mg ml⁻¹) in aluminium hydroxide (Al(OH)₃) moist gel. As a concurrent sham-immunized control, littermates were injected with Al(OH)₃ alone (30 mg in 0.5 ml saline). Antigen and/or adjuvant administration was repeated weekly for the first month and then biweekly for the next two months in immunized and sham-immunized rabbits, respectively. In other experiments, naive animals were kept for 3 months. The methodology described in this study was subject to Home Office approval and performed under the Animals (Scientific Procedures) Act 1986.

At 3 months of age, the animals were transferred from the breeding unit to our laboratory, where they were skin tested to determine their sensitivity to the antigen.

Pulmonary function measurements

Rabbits (1.8–3.7 kg) were pre-medicated with diazepam (5 mg ml⁻¹, 5 mg kg⁻¹ i.p.) and subsequently anaesthetized with Hypnorm (0.4 ml kg⁻¹, i.m.; a mixture of fentanyl citrate 0.315 mg ml⁻¹ and fluanisone 10 mg ml⁻¹), a regime which produces neuroleptanalgesia and is recommended for recovery anaesthesia in laboratory rabbits (Flecknall, 1987). Animals were placed in a supine position and intubated with a 3.0 mm endotracheal tube (Mallinckrodt Laboratories, Athlone, Ireland). The cuff was then inflated and the tube attached to a heated Fleisch pneumotachograph (size 00). Flow was measured with a Validyne differential pressure transducer (model MP 45-14-871; Validyne Engineering Corp., Northridge, C.A.). Pleural pressure was estimated by placing an oesophageal balloon in the lower third of the oesophagus to obtain the maximum expiratory pressure. Transpulmonary pressure, the difference between thoracic and pleural pressure, was measured with another Validyne differential pressure transducer (model MP 45-24-871) connected between the outflow of the endotracheal tube and the oesophageal balloon. The flow was integrated to obtain a continuous recording of tidal volume. Measurements of total lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated by an online respiratory analyser (PMS Version 4.0, Mumed Ltd., London) as previously described (Spina *et al.*, 1991). Neuroleptanalgesia was maintained throughout the course of the experiment by administration of Hypnorm i.m. approximately every 30 min (Flecknall, 1987).

Measurement of airway responsiveness to histamine

After measurement of baseline lung function, rabbits were exposed to an aerosol of sterile saline for 2 min and lung

function parameters recorded. Airway responsiveness was determined by exposing animals to cumulative concentrations of aerosolized histamine (1.25–80 mg ml⁻¹; 2 min per concentration) administered directly to the lungs via an endotracheal tube. Pulmonary function was recorded following each 2 min exposure.

Aerosols were generated by an ultrasonic nebulizer (Devilbiss Health Care, UK Ltd., Hounslow, Middlesex) which has previously been demonstrated to generate particles of which the majority are in the 0.5–5 µm diameter range.

The provocation concentration (PC) of histamine that produced a 50% increase in R_L (PC₅₀) and 35% decrease in C_{dyn} (PC₃₅) was determined for each animal and used as indices of airway responsiveness.

PAF challenge and drug administration

On day 2, animals were re-anaesthetized and challenged with either PAF or 0.25% bovine serum albumin (BSA) (the carrier molecule for PAF). After exposure to an aerosol of BSA for 2 min, rabbits were exposed to PAF (80 µg ml⁻¹) or BSA over a 1 h period, after which time respiratory parameters were then recorded. Control animals received a 2 min aerosol of BSA followed by a 60 min aerosol of BSA containing the same concentration of ethanol as that received by the PAF-treated animals (approximately 1%).

On days 3, 5 and 9, increasing concentrations of histamine were administered to the anaesthetized rabbits as on day 1 and the PC₅₀ (R_L) and the PC₃₅ (C_{dyn}) values determined.

Drug studies

A suspension of PF 5901 (0.5 mg, 1 mg, 3 mg or 10 mg in a volume of 0.5 ml) or vehicle was instilled directly into the lung via a cannula passed into the airways, to the point of the bifurcation, via the endotracheal tube, 1 h before the start of the PAF aerosol. Similarly, a suspension of PF 5901 (10 mg) was instilled into the airways 1 h before a corresponding aerosol of 0.25% BSA. In these experiments respiratory parameters were recorded prior to, and 1, 15, 30 and 60 min following the drug administration, then as previously described for the BSA challenge.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed immediately after completion of airway responsiveness to histamine. The airways were lavaged by use of a polyethylene catheter inserted into the lung via the endotracheal tube. Five ml of 0.9% sterile saline was injected into the lungs then immediately aspirated, with 50% recovery of BAL fluid. Total cell counts were determined under light microscopy using an improved Neubauer haemocytometer. For differential cell counts, 25 µl aliquots were used for centrifugation (Shandon cytospin 2; Shandon Southern Instruments, Sewickley, PA, U.S.A.) and the cells were stained with Lendrum's stain (haematoxylin and chromotrope 2R) to facilitate the discrimination of eosinophils. A total of 200 cells were counted differentially and classified as either neutrophils, eosinophils or mononuclear cells based on standard morphological criteria. All cytospins were counted 'blind'.

Analysis of results

Results of the lung function studies are expressed as mean ± s.e.mean. *In vivo* histamine potency values were derived from measurements of airway resistance (R_L) (PC₅₀) and dynamic compliance (C_{dyn}) (PC₃₅) and are expressed as the geometric means together with upper and lower values for s.e.mean. For statistical purposes PC₅₀ and PC₃₅ values were log₁₀ transformed.

One-way analyses of variance were used to analyse the bronchoconstriction data (R_L and C_{dyn}) (expressed as percen-

tage change). Two-way analyses of variance for repeated measures were employed to analyse the histamine potency data before and at the 3 time points after PAF administration. The total cell counts and mononuclear cell counts obtained from BAL before and 24 h, 72 h and 1 week after PAF challenge were subjected to the same analysis. Eosinophil and neutrophil counts before and after PAF challenge were analyzed by a Friedman two-way analysis of variance by ranks (Chi-squared determination) as the variances were found to be non-homogenous. A requirement of this analysis is equal group sizes therefore missing values were estimated (Winer, 1971). Where appropriate, Tukey's HSD test or distribution-free multiple comparisons were used to determine differences in means when multiple comparisons were made. Results were considered significant if $P < 0.05$.

Drugs

Alternaria tenuis extract was obtained from Greer Laboratories Inc. Lenoir, N.C., U.S.A. (40,000 PNU ml⁻¹, 1 mg ml⁻¹) and aluminium hydroxide (Al(OH)₃) moist gel from FSA Laboratory supplies, Loughborough, U.K. Histamine diphosphate and bovine serum albumin (low endotoxin) were obtained from Sigma (Poole, Dorset); PAF was purchased from Novabiochem (Nottingham, U.K.); diazepam (Valium, Roche, U.K.); fentanyl citrate (Hypnorm, Janssen Pharmaceutical Ltd., U.K.); PF 5901 (2-[3-(1-hydroxyhexyl)phenoxymethyl]quinoline hydrochloride) (The Purdue Frederick Company, Norwalk, Connecticut, U.S.A.) (100 mg) was dissolved in 2 ml polyethylene glycol 400 and diluted with 0.9% saline as required.

Results

Airway responsiveness in vivo

No significant difference was observed between airway responsiveness (airway resistance R_L or dynamic compliance C_{dyn}) to inhaled histamine at 3 months in rabbits neonatally immunized with the antigen *Alternaria tenuis* and those rabbits sham-immunized only (Tables 1A and B). The increased responsiveness of both immunized and sham-immunized rabbits to inhaled histamine compared with naive rabbits did not reach statistical significance (Tables 1A and B).

Effect of PAF

Acute bronchospasm In the 3 animal groups i.e. allergen-immunized, sham-immunized and naive, a 1 h aerosol of PAF caused an increase in airway resistance and a decrease in dynamic compliance to a similar extent (Figure 1). This bronchoconstrictor response to PAF had resolved by the time of the next lung function measurement i.e. by 24 h (data not shown). The administration of an aerosol of 0.25% BSA to immunized rabbits caused changes in R_L and C_{dyn} that were significantly smaller than those changes elicited in the other 3 animal groups (Figure 1).

Airway hyperresponsiveness At 24 h following PAF administration in immunized rabbits, airway responsiveness to inhaled histamine (R_L and C_{dyn}) was significantly increased compared to pre-PAF levels (Tables 1A and B). This PAF-induced hyperresponsiveness was still evident 72 h after PAF administration (Tables 1A and B). Both the R_L and C_{dyn} components had returned to pre-PAF levels 1 week following PAF challenge (Tables 1A and B). In the sham-immunized and normal groups, PAF did not significantly alter airway responsiveness to inhaled histamine at 24 h, 72 h or 1 week following challenge when considering either the R_L or C_{dyn} component (Tables 1A and B). The individual responses (histamine PC_{50} and PC_{35}) of immunized, sham-immunized and normal rabbits before and 24 h after PAF challenge are

Table 1 Airway responsiveness to histamine prior to, and 24 h, 72 h and 1 week following PAF challenge (80 µg ml⁻¹) in immunized, sham-immunized and normal rabbits: the effect of 0.25% bovine serum albumin (vehicle for PAF) on airway responsiveness to histamine in immunized rabbits is also represented.

A		Histamine PC_{50} (mg ml ⁻¹)			
		Pre	24 h	72 h	1 week
Immunized/PAF	<i>n</i>	10	10	7	6
	mean	11.54	3.44*	3.88*	14.39
	s.e.mean	1.20	1.26	1.47	1.91
Immunized/BSA	<i>n</i>	4	4	3	3
	mean	11.69	10.33	12.30	8.71
	s.e.mean	1.95	1.56	1.50	1.61
Sham/PAF	<i>n</i>	10	10	10	8
	mean	13.43	16.48	10.81	7.12
	s.e.mean	1.42	1.53	1.32	1.39
Normal/PAF	<i>n</i>	10	10	10	9
	mean	16.71	10.94	10.79	12.33
	s.e.mean	1.37	1.42	1.33	1.46

B		Histamine PC_{35} (mg ml ⁻¹)			
		Pre	24 h	72 h	1 week
Immunized/PAF	<i>n</i>	10	10	7	6
	mean	14.87	4.58*	6.32*	9.61
	s.e.mean	1.29	1.02	1.50	1.70
Immunized/BSA	<i>n</i>	4	4	3	3
	mean	11.17	6.59	7.05	4.90
	s.e.mean	1.85	1.66	1.12	1.30
Sham/PAF	<i>n</i>	10	10	10	8
	mean	9.14	5.35	7.35	8.23
	s.e.mean	1.27	1.31	1.45	1.38
Normal/PAF	<i>n</i>	10	10	10	9
	mean	25.76	18.79	13.18	10.74
	s.e.mean	1.59	1.46	1.36	1.59

(a) Histamine PC_{50} is the concentration of histamine (aerosol) (mg ml⁻¹) required to cause a 50% increase in airway resistance (R_L); (b) Histamine PC_{35} is the concentration of histamine (aerosol) (mg ml⁻¹) required to cause a 35% fall in dynamic compliance (C_{dyn}). PAF treatment significantly increased airway responsiveness to histamine at 24 h and 72 h following challenge in immunized rabbits (* $P < 0.05$).

Figure 1 (a) Percentage change in airway resistance (R_L) and dynamic compliance (C_{dyn}) following PAF aerosol (80 µg ml⁻¹) in immunized (solid columns), sham-immunized (cross-hatched columns) and normal (hatched columns) rabbits. Also shown is R_L and C_{dyn} following BSA aerosol in immunized rabbits (open columns). * $P < 0.05$ compared with PAF aerosol. (b) Percentage change in airway resistance (R_L) and dynamic compliance (C_{dyn}) following PAF aerosol (80 µg ml⁻¹) in immunized rabbits pretreated with PF 5901 vehicle (solid columns), 0.5 mg (right diagonal columns), 1 mg (cross-hatched columns), 3 mg (left diagonal columns) and 10 mg (open columns).

* $P < 0.05$ compared with vehicle control.

presented in Figures 2a and b. In contrast to the sham-immunized and normal rabbits, every immunized rabbit developed airway hyperresponsiveness to histamine 24 h after PAF. Exposure of 0.25% BSA (1 h aerosol) to immunized rabbits did not alter airway responsiveness to histamine at any of the measured time points (Tables 1A and B).

Effect of PF 5901 in allergen-immunized rabbits

Airway responsiveness Baseline airway responsiveness (both R_L and C_{dyn}) to inhaled histamine in immunized rabbits was not significantly different in groups that received either vehicle only, PF 5901 0.5 mg, 1 mg, 3 mg or 10 mg (Tables 2A and B). The combined mean values were R_L : $PC_{50} = 11.6 \pm 1.2 \text{ mg ml}^{-1}$ ($n = 30$); $C_{dyn} = 10.0 \pm 1.1 \text{ mg ml}^{-1}$ ($n = 30$).

Baseline lung function Single doses of vehicle or PF 5901 10 mg instilled directly into the lungs of immunized rabbits had no significant effect on baseline lung function (R_L or C_{dyn}) measured 1, 15, 30 or 60 min following administration (data not shown; $n = 4$).

PAF-induced bronchoconstriction Direct intratracheal instillation of the vehicle for PF 5901 did not affect PAF-induced bronchoconstriction compared with untreated rabbits ($R_L + 83.6 \pm 34.2\%$ ($n = 5$) and $+63.8 \pm 16.1\%$ ($n = 10$), respectively; $C_{dyn} - 43.0 \pm 6.5\%$ ($n = 5$) and $-44.5 \pm 7.5\%$ ($n = 10$), respectively). Bronchoconstriction induced by PAF was not significantly different in groups of allergen-immunized rabbits pretreatment with vehicle or with PF 5901 at doses of 0.5 mg, 1.0 mg and 3 mg directly administered into the airway (Figure 1b). PF 5901 10 mg however,

Table 2 The effect of PF 5901 (vehicle, 0.5 mg, 1 mg, 3 mg and 10 mg) on airway responsiveness to histamine prior to, and 24 h, 72 h and 1 week following PAF challenge ($80 \mu\text{g ml}^{-1}$) in immunized rabbits

A	PF 5901		Histamine PC_{50} (mg ml^{-1})			
			Pre	24 h	72 h	1 week
Vehicle	<i>n</i>		6	6	6	6
	mean		12.76	4.06*	5.82*	7.79
	s.e.mean		1.52	1.41	1.59	1.63
0.5 mg	<i>n</i>		6	6	6	5
	mean		14.85	6.22*	5.66*	7.15
	s.e.mean		1.02	1.58	1.43	1.40
1 mg	<i>n</i>		6	6	4	4
	mean		9.04	10.74	6.84	10.89
	s.e.mean		1.46	1.56	1.27	1.38
3 mg	<i>n</i>		6	6	6	6
	mean		10.74	14.52	25.76	12.19
	s.e.mean		1.32	1.64	1.84	1.41
10 mg	<i>n</i>		6	6	6	6
	mean		11.54	11.51	11.72	8.09
	s.e.mean		1.56	1.67	1.02	1.43

B	PF 5901		Histamine PC_{35} (mg ml^{-1})			
			Pre	24 h	72 h	1 week
Vehicle	<i>n</i>		6	6	6	6
	mean		11.25	3.59*	6.71	5.31
	s.e.mean		1.02	1.37	1.56	1.42
0.5 mg	<i>n</i>		6	6	6	5
	mean		13.93	5.83*	8.61	7.78
	s.e.mean		1.36	1.60	1.36	1.02
1 mg	<i>n</i>		6	6	4	4
	mean		7.24	7.80	5.02	8.07
	s.e.mean		1.46	1.46	1.10	1.37
3 mg	<i>n</i>		6	6	6	6
	mean		10.91	13.27	16.63	8.87
	s.e.mean		1.30	1.51	1.51	1.44
10 mg	<i>n</i>		6	6	6	6
	mean		7.83	8.57	8.17	5.11
	s.e.mean		1.33	1.70	1.37	1.42

(a) Histamine PC_{50} is the concentration of histamine (aerosol) (mg ml^{-1}) required to cause a 50% increase in airway resistance (R_L); (b) Histamine PC_{35} is the concentration of histamine (aerosol) (mg ml^{-1}) required to cause a 35% fall in dynamic compliance (C_{dyn}).

* $P < 0.05$ compared with Pre value

significantly inhibited the R_L component of the PAF-induced bronchoconstriction ($P < 0.05$) without exhibiting a significant inhibitory effect on C_{dyn} (Figure 1b).

Effect of PF 5901 treatment on PAF-induced airway hyperresponsiveness

Airway responses to inhaled histamine (PC_{50} or PC_{35}) were not significantly different in animals treated with PF 5901 10 mg 1 h before BSA aerosol at 24 h, 72 h or 1 week following challenge, compared with pre-BSA values (data not shown). Single doses of 1 mg, 3 mg and 10 mg PF 5901 directly instilled into the lungs of allergen-immunized rabbits were sufficient to inhibit significantly PAF-induced airway hyperresponsiveness at 24 h following PAF challenge for both R_L and C_{dyn} , compared with the vehicle-treated control group ($P < 0.05$) (Tables 2A and B, Figures 3a and b).

Brochoalveolar lavage

Effect of PAF on cell counts in bronchoalveolar lavage Before PAF exposure, mononuclear cells comprised more than 95% of the resident cell population with a small (less than 5%) number of neutrophils (Table 3). The percentage of neutrophils, eosinophils and mononuclear cells recovered

Figure 2 Individual histamine (a) PC_{50} (R_L) and (b) PC_{35} (C_{dyn}) values before and 24 h after aerosol exposure of PAF ($80 \mu\text{g ml}^{-1}$) in immunized (●), sham-immunized (▼) and normal (■) rabbits.

Figure 3 Effect of PF 5901 vehicle (□) ($n = 6$) and PF 5901 10 mg (●) ($n = 6$) on PAF-induced airway hyperresponsiveness in immunized rabbits: (a) histamine PC₅₀ is the concentration of histamine required to cause a 50% increase in airway resistance (R_L); (b) histamine PC₃₅ is the concentration of histamine required to cause a 35% decrease in dynamic compliance (C_{dyn}). * $P < 0.05$ compared with pre-PAF control.

Table 3 Percentage of neutrophils, eosinophils and mononuclear cells recovered from bronchoalveolar lavage (BAL) in immunized, sham-immunized and normal rabbits

	% cells		
	Neutrophils	Eosinophils	Mononuclear cells
Immunized ($n = 7$)	3.21 ± 0.65	0.93 ± 0.34	95.71 ± 0.77
Sham-immunized ($n = 9$)	2.83 ± 0.82	0.59 ± 0.24	96.67 ± 0.88
Normal ($n = 10$)	4.30 ± 0.97	0.10 ± 0.10	95.60 ± 1.01

Values are mean $\pm$ s.e.mean.

from BAL prior to PAF exposure in immunized, sham-immunized and normal rabbits is shown in Table 3.

The mean pretreatment cell counts from the 3 groups of rabbits i.e. immunized, sham-immunized and normals were not significantly different (Table 4). Total leukocyte counts were significantly elevated in the airways as assessed by BAL 24 h after PAF exposure in immunized, sham-immunized and normal rabbits ($P < 0.05$) (Table 4). Total cell counts remained elevated at 72 h following PAF in the immunized and normal rabbit groups ($P < 0.05$) (Table 4). By 1 week, counts had returned to pre-PAF levels (Table 4) in all groups.

Although there was an increase in the number of mononuclear cells in BAL 24 h and/or 72 h after PAF challenge in all 3 animal groups, this was not statistically significant. In contrast, a significant increase in the number of BAL neutrophils and eosinophils was observed at 24 h and 72 h following PAF in immunized, sham-immunized and normal rabbits ($P < 0.05$).

Exposure to immunized rabbits of an aerosol of 0.25% BSA induced a small but insignificant cellular infiltrate into the airways. However, there was a significant increase in the number of neutrophils present in the airways 24 h following BSA ($P < 0.05$) (Table 5).

Effect of PF 5901 treatment on PAF-induced changes in cell counts in BAL

The PAF-induced cellular infiltration profile for both total and differential cell counts observed in control rabbits treated with vehicle was not significantly different from that observed in rabbits treated with PF 5901 at any dose (Table 6).

Table 4 Number of total cells and differential cell counts recovered from bronchoalveolar lavage (BAL) before (Pre) and 24 h, 72 h and 1 week following exposure to PAF ($80 \mu\text{g ml}^{-1}$) in immunized, sham-immunized and normal rabbits

	$\times 10^5 \text{ cells ml}^{-1}$			
	Total	Neutrophils	Eosinophils	Mononuclear cells
Immunized				
Pre ($n = 7$)	1.03 ± 0.28	0.031 ± 0.009	0.006 ± 0.003	0.99 ± 0.28
24 h ($n = 7$)	$4.19 \pm 0.57^*$	$1.120 \pm 0.330^*$	$0.241 \pm 0.106^*$	2.34 ± 0.56
72 h ($n = 7$)	$2.66 \pm 0.59^*$	$0.760 \pm 0.230^*$	$0.090 \pm 0.027^*$	1.74 ± 0.42
1 week ($n = 5$)	1.17 ± 0.42	0.213 ± 0.056	0.014 ± 0.006	0.94 ± 0.38
Sham-immunized				
Pre ($n = 8$)	1.04 ± 0.39	0.024 ± 0.014	0.008 ± 0.004	1.01 ± 0.38
24 h ($n = 8$)	$3.63 \pm 0.87^*$	$1.630 \pm 0.520^*$	$0.187 \pm 0.044^*$	1.82 ± 0.45
72 h ($n = 6$)	2.13 ± 0.61	$0.860 \pm 0.273^*$	$0.173 \pm 0.087^*$	1.09 ± 0.31
1 week ($n = 6$)	2.02 ± 0.55	0.439 ± 0.157	$0.138 \pm 0.067^*$	1.44 ± 0.40
Normal				
Pre ($n = 10$)	1.28 ± 0.23	0.056 ± 0.153	0.001 ± 0.001	1.22 ± 0.23
24 h ($n = 10$)	$3.09 \pm 0.52^*$	$1.880 \pm 0.441^*$	$0.160 \pm 0.038^*$	1.04 ± 0.16
72 h ($n = 10$)	$4.43 \pm 1.35^*$	$1.847 \pm 0.594^*$	$0.302 \pm 0.189^*$	2.27 ± 0.69
1 week ($n = 8$)	3.09 ± 1.12	$0.961 \pm 0.302^*$	$0.260 \pm 0.159^*$	1.87 ± 0.69

Results are expressed as mean $\pm$ s.e.mean.

PAF exposure significantly increased the total number of cells and the number of neutrophils and eosinophils recovered in BAL cf. Pre PAF levels in all 3 rabbit groups (* $P < 0.05$).

Table 5 Number of total cells and differential cell counts recovered from bronchoalveolar lavage (BAL) in immunized rabbits before (Pre) and 24 h, 72 h and 1 week following exposure to bovine serum albumin (BSA 0.25%)

	$\times 10^5 \text{ cells ml}^{-1}$			
	Total	Neutrophils	Eosinophils	Mononuclear cells
Pre ($n = 4$)	1.13 ± 0.48	0.22 ± 0.16	0.003 ± 0.002	0.90 ± 0.33
24 h ($n = 4$)	2.38 ± 0.95	$1.17 \pm 0.67^*$	0.031 ± 0.009	1.17 ± 0.33
72 h ($n = 3$)	1.38 ± 0.29	0.61 ± 0.16	0.050 ± 0.016	0.72 ± 0.19
1 week ($n = 3$)	1.20 ± 0.40	0.23 ± 0.51	0.040 ± 0.029	0.93 ± 0.34

Results are expressed as mean $\pm$ s.e.mean.

BSA exposure significantly increased the total number of neutrophils recovered in BAL cf. Pre BSA levels (* $P < 0.05$).

Table 6 Number of total cells and differential cell counts recovered from bronchoalveolar lavage (BAL) before (Pre) and 24 h, 72 h and 1 week following exposure to PAF ($80 \mu\text{g ml}^{-1}$) in immunized rabbits pretreated with PF 5901 0 mg, 0.5 mg, 1 mg, 3 mg and 10 mg

		$\times 10^5 \text{ cells ml}^{-1}$			
		Total	Neutrophils	Eosinophils	Mononuclear cells
0 mg (vehicle)	Pre ($n = 6$)	0.99 ± 0.18	0.076 ± 0.017	0.003 ± 0.002	0.91 ± 0.18
	24 h ($n = 6$)	$2.39 \pm 0.46^*$	$0.982 \pm 0.131^*$	$0.235 \pm 0.118^*$	1.17 ± 0.31
	72 h ($n = 6$)	2.98 ± 0.86	$1.740 \pm 0.770^*$	$0.151 \pm 0.037^*$	1.07 ± 0.19
	1 week ($n = 6$)	1.60 ± 0.32	$0.383 \pm 0.065^*$	$0.154 \pm 0.465^*$	1.06 ± 0.26
0.5 mg	Pre ($n = 6$)	1.03 ± 0.23	0.121 ± 0.073	0.002 ± 0.002	0.98 ± 0.22
	24 h ($n = 6$)	$5.27 \pm 1.42^*$	$3.368 \pm 0.960^*$	$0.340 \pm 0.120^*$	1.50 ± 0.55
	72 h ($n = 6$)	3.37 ± 1.69	$2.404 \pm 1.699^*$	$0.161 \pm 0.070^*$	0.99 ± 0.28
	1 week ($n = 4$)	1.81 ± 0.74	$0.695 \pm 0.230^*$	$0.087 \pm 0.027^*$	1.03 ± 0.50
1 mg	Pre ($n = 6$)	0.98 ± 0.20	0.059 ± 0.036	0.012 ± 0.004	0.88 ± 0.14
	24 h ($n = 5$)	$3.52 \pm 0.84^*$	$2.194 \pm 0.598^*$	$0.358 \pm 0.178^*$	0.97 ± 0.22
	72 h ($n = 4$)	1.26 ± 0.19	$0.412 \pm 0.103^*$	$0.049 \pm 0.014^*$	0.80 ± 0.19
	1 week ($n = 3$)	1.15 ± 0.24	0.270 ± 0.066	0.005 ± 0.005	0.88 ± 0.18
3 mg	Pre ($n = 5$)	1.16 ± 0.39	0.045 ± 0.010	0.013 ± 0.010	1.10 ± 0.37
	24 h ($n = 4$)	$4.43 \pm 1.32^*$	$3.153 \pm 0.941^*$	$0.179 \pm 0.102^*$	1.09 ± 0.30
	72 h ($n = 5$)	1.76 ± 0.59	$0.688 \pm 0.331^*$	$0.081 \pm 0.016^*$	0.92 ± 0.26
	1 week ($n = 4$)	1.54 ± 0.48	$0.407 \pm 0.135^*$	$0.152 \pm 0.092^*$	0.98 ± 0.33
10 mg	Pre ($n = 4$)	1.03 ± 0.17	0.015 ± 0.005	0.003 ± 0.010	0.82 ± 0.26
	24 h ($n = 4$)	$3.53 \pm 0.33^*$	$1.084 \pm 0.190^*$	$0.445 \pm 0.024^*$	1.20 ± 0.42
	72 h ($n = 4$)	1.15 ± 0.31	$0.323 \pm 0.161^*$	$0.090 \pm 0.046^*$	0.74 ± 0.15
	1 week ($n = 4$)	0.60 ± 0.15	0.034 ± 0.012	$0.021 \pm 0.005^*$	0.55 ± 0.16

Results are expressed as mean $\pm$ s.e.mean.

PAF exposure significantly increased the total number of cells and the number of neutrophils and eosinophils recovered in BAL cf. Pre PAF levels in rabbits pretreated with vehicle or with PF 5901 (any dose) (* $P < 0.05$).

Discussion

The current study has demonstrated that aerosolized PAF will induce acute bronchospasm in normal rabbits and rabbits that have been neonatally immunized with antigen. It is of interest that the degree of acute bronchoconstriction induced by PAF in the immunized rabbits is not significantly different from that induced in the sham-immunized or normal rabbits. This agrees with studies in man, where subjects with bronchial asthma are known to be hyperresponsive to inhaled histamine when compared with normals, yet asthmatics appear not to be hyperresponsive to inhaled PAF (Cuss *et al.*, 1986; Rubin *et al.*, 1987; Chung & Barnes, 1989). Furthermore, PAF-induced bronchoconstriction in the rabbit appears to be partly mediated via the release of 5-lipoxygenase products since it can be significantly reduced by pretreatment with PF 5901. The significant inhibition of the resistance component of the PAF-induced bronchoconstriction only may suggest that the contribution of lipoxygenase products to the alterations induced by PAF in the large airways is greater than that in the smaller airways. These results support observations in man that cysteinyl-leukotriene antagonists inhibit not only bronchoconstriction induced by PAF (Spencer *et al.*, 1990a; Kidney *et al.*, 1991), but also that induced by antigen (Taylor *et al.*, 1991) and by cold air (Israel *et al.*, 1991). The cellular source of these lipoxygenase

products remains to be determined, but is unlikely to be airway muscle itself since PAF does not contract human bronchial smooth muscle directly (Schellenberg *et al.*, 1983), and there appears to be only a low concentration of PAF receptors located on human airway smooth muscle (Goldie *et al.*, 1990).

A significant difference in airway responsiveness to histamine between immunized and sham-immunized rabbits has been previously reported (McKenniff *et al.*, 1991). This finding is based on large numbers of animals as there is considerable overlap between the groups. However, in the present experiments no significant difference in airways responsiveness was observed between immunized and sham-immunized animals, suggesting that the adjuvant alone may induce airway hyperresponsiveness. This lack of significant difference between the groups may be due to the relatively small numbers of animals used.

The present study has shown that aerosolised PAF will induce bronchial hyperresponsiveness to inhaled histamine in some, but not all normal rabbits, supporting previous studies (Coyle *et al.*, 1990). However, interestingly, PAF induced bronchial hyperresponsiveness to inhaled histamine in all neonatally immunized rabbits to some degree. PAF-induced hyperresponsiveness lasted for 72 h but had returned to baseline in the majority of animals by one week. The mechanisms(s) underlying the ability of PAF to induce bron-

chial hyperresponsiveness to a greater degree in immunized rather than normal or sham-immunized rabbits is unknown, but PAF has previously been reported to elicit eosinophil infiltration in the skin of allergic subjects but not in normal subjects (Henocq & Vargaftig, 1988; Fadel *et al.*, 1990), and has been reported to elicit a greater degree of eosinophils infiltration into the lungs of actively immunized guinea-pigs when compared with normal guinea-pigs (Sanjar *et al.*, 1989). However, in our study there was no significant difference observed between the number of inflammatory cells recruited into bronchoalveolar lavage fluid from normal, sham-immunized and immunized rabbits following exposure to PAF. Nonetheless, the inflammatory cells resident in the airway of the neonatally immunized rabbit may be primed as described in other situations (Frigas & Gleich, 1986) and therefore more readily release their stored and preformed cytotoxic mediators in response to PAF stimulation.

The ability of PAF to induce bronchial hyperresponsiveness in the neonatally immunized rabbit was significantly attenuated in a dose-related manner by prior treatment with PF 5901. As this drug had no effect on the airway responsiveness to histamine following BSA challenge, this inhibitory action of PF 5901 is not attributable to histamine H_1 antagonism or via some non-specific effect on airway hyperresponsiveness. PF 5901 was originally described as a 5-lipoxygenase inhibitor (Van Inwegen *et al.*, 1987) and is now recognized as being a 5-lipoxygenase-activating protein (FLAP) inhibitor similar to the prototype FLAP inhibitor MK 886 (Evans *et al.*, 1991). Following cell stimulation, leucocyte 5-lipoxygenase is translocated from its cytosolic location to a cell membrane site (Rouzer & Kargman, 1988) where it is fully activated by FLAP, an 18,000-D membrane protein. Full expression of 5-lipoxygenase and FLAP is required in cells to catalyze the C-5 oxygenation of arachidonic acid to generate 5-HPETE, the key intermediate in the formation of leukotrienes (Samuelsson *et al.*, 1987). PF 5901 is also a moderately potent antagonist of LTD₄ (Van Inwegen *et al.*, 1987).

Of particular interest is the recent finding that PF 5901 will also inhibit PAF synthesis in rodent mast cells (Hogaboam *et al.*, 1992). In recent studies, LTC₄, LTD₄ and LTE₄ have been shown to increase airway responsiveness to histamine in asthmatic but not normal subjects (Arm *et al.*, 1988; O'Hickey *et al.*, 1991). Furthermore, in atopic subjects the LTD₄ receptor antagonist ICI 204.219 was found to inhibit significantly antigen-induced airway hyperresponsiveness in response to histamine (Taylor *et al.*, 1991). Which of the actions of PF 5901 is responsible for the inhibition of PAF-induced bronchial hyperresponsiveness has not yet been determined, but it is perhaps of relevance that we have previously reported that capsaicin will also inhibit PAF-induced bronchial hyperresponsiveness in the rabbit, through a mechanism unrelated to its ability to deplete sensory

neuropeptides (Spina *et al.*, 1991) and that capsaicin has been reported to inhibit 5-lipoxygenase activity in human neutrophils (Flynn *et al.*, 1986).

In agreement with previous studies, PAF was found to induce an influx of inflammatory cells (predominantly neutrophils and eosinophils) into the airways as assessed by bronchoalveolar lavage (BAL) (Coyle *et al.*, 1990; Spina *et al.*, 1991). The accumulation and activation of inflammatory cells within the airways has been suggested to lead to epithelial damage causing the exposure of nerve endings in the bronchial lumen, which are thought to initiate an increase in airway responsiveness (Barnes, 1986). In the present study we have shown that this effect of PAF on cellular infiltration is inducible in normal, sham-immunized and immunized rabbits to a similar extent. In general, the recruitment of these cells persists within the lung for at least 3 days following PAF exposure, with baseline levels returning by one week.

However, PF 5901 did not inhibit PAF-induced cellular infiltration into the airways as assessed by BAL, despite inhibiting the associated bronchial hyperresponsiveness. This finding is supportive of other experiments performed in this model where pretreatment of neonatally immunized rabbits with capsaicin inhibits bronchial hyperresponsiveness but not the pulmonary eosinophil infiltration induced by PAF (Spina *et al.*, 1991). Furthermore, in guinea-pigs, capsaicin will inhibit antigen-induced bronchial hyperresponsiveness without modifying eosinophil infiltration (Ladenius & Biggs, 1989; Matsuse *et al.*, 1991) and cytokines can cause pulmonary eosinophilia without bronchial hyperresponsiveness (Kings *et al.*, 1990). Low dose antigen in guinea-pigs has been reported to induce eosinophil accumulation in BAL in the absence of any alteration in airways responsiveness, and at a higher dose of antigen, ketotifen, AH 21132, dexamethasone and aminophylline were shown to inhibit the pulmonary eosinophilia and not the associated airway hyperresponsiveness (Sanjar *et al.*, 1990). Together these results suggest that the presence of eosinophils within the airway lumen may not be a prerequisite for the development of bronchial hyperresponsiveness. Such an interpretation is consistent with recent clinical observations that bronchial hyperresponsiveness may be present in asthmatics without an observed eosinophil infiltrate (Lundgren *et al.*, 1988) and conversely, chronic eosinophilic bronchitis is not always associated with airway hyperresponsiveness (Gibson *et al.*, 1989). However, in the absence of data on the activation state of the eosinophils present in the vehicle- and PF 5901-treated groups, caution must be exercised in ruling out the participation of eosinophils at this time.

In conclusion therefore, PAF induces long-lasting bronchial hyperresponsiveness in neonatally immunized rabbits which can be inhibited by PF 5901, an effect that may not be related to the associated pulmonary infiltration of inflammatory cells.

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