Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs

¹S. Sanjar, ²S. Aoki, A. Kristersson, ³D. Smith & J. Morley

Preclinical Research, Sandoz Ltd, CH-4002 Basel, Switzerland

- 1 Guinea-pigs were sensitized with 3 injections of ovalbumin (OA) (1 or 10 µg per animal) using Al(OH)₃ and pertussis vaccine as adjuvants at two week intervals.
- 2 Sensitized guinea-pigs were challenged with an aerosol of OA (0.1%) over a one hour period and both airway reactivity and cellular content of bronchoalveolar lavage (BAL) fluid were assessed at intervals for up to 7 days.
- 3 Guinea-pigs sensitized with 1 µg of ovalbumin responded to an aerosol of OA with increased pulmonary airway eosinophilia, which was evident 1 day after challenge and was present for up to 7 days. Airway hyperreactivity was not detectable in these animals.
- 4 Guinea-pigs sensitized with 10 µg of ovalbumin responded to an aerosol of OA with increased pulmonary airway neutrophilia and eosinophilia and with increased airway reactivity which was maximal between 8 and 24 h after exposure to OA.
- 5 Depletion of circulating platelets or neutrophils, by use of selective antisera, did not alter either the magnitude of eosinophilia or the intensity of airway reactivity in sensitized guinea-pigs (10 µg) exposed to an aerosol of OA.
- 6 Pretreatment of sensitized guinea-pigs (10 µg) for 6 days with AH 21-132, aminophylline, dexamethasone or ketotifen inhibited pulmonary airway eosinophilia, but did not diminish airway hyperreactivity. Neither eosinophil accumulation nor development of airway hyperreactivity was influenced by treatment with mepyramine or salbutamol over a 6 day period before OA inhalation.
- 7 Although eosinophilia may occur in association with increased airway reactivity in this animal model, there is no evidence of a causal relationship.

Introduction

The presence of eosinophils in airways of asthma patients was recognised over sixty years ago in histological preparations of lung tissue (Huber & Koessler, 1922). Increased eosinophil numbers in peripheral blood, sputum or lung tissue are now widely acknowledged to be characteristic of asthma (Dunnill, 1960; Epstein, 1972; Horn *et al.*, 1975; Frigas & Gleich, 1986). In addition, toxic eosinophil products, such as major basic protein (MBP) (Frigas *et al.*, 1981) and eosinophil cationic protein (ECP) (Dahl *et al.*, 1978) have been demonstrated in the sputum and blood of asthma patients. Further evidence of eosinophil involvement in inflammatory reactions within the asthmatic lung has been provided, by demonstration of an increased bronchial eosinophilia in patients exhibiting a late asthmatic response (De Monchy *et al.*, 1985) and of a positive correlation between airway reactivity to methacholine and blood eosinophilia in asthmatics who respond to allergen with a biphasic airway obstruction (Durham & Kay, 1985). Because of this association, it is widely considered that airway eosinophilia leads to epithelial desquamation as a consequence of MBP and ECP release, which in turn would lead to airway hyperreactivity (Flavahan *et al.*, 1988).

The present investigation has sought to study the relationship between airway eosinophilia and hyperreactivity in sensitized guinea-pigs after exposure to antigen. The guinea-pig has been chosen as a test animal because of histological simi-

larities that exist between lungs of antigen exposed guinea-pigs and asthmatic lungs in man (reviewed by Kallos & Kallos, 1984) and because this species can exhibit early and late-onset airway obstruction (Hutson *et al.*, 1988a), bronchial eosinophilia (Aoki *et al.*, 1988; Dunn *et al.*, 1988) and increased airway reactivity following exposure of sensitized animals to antigen (Aoki *et al.*, 1988; Daffonchio *et al.*, 1989). The effect of a number of established anti-asthma drugs, and a novel substance AH 21-132 (Bewley & Chapman, 1988; Sanjar *et al.*, 1990), upon antigen-induced airway eosinophilia and hyperreactivity has been studied. In addition, the role of circulating neutrophils and platelets in the development of these events has been assessed by use of lytic antisera for selective depletion of these cells.

Methods

Animals

Male Dunkin-Hartley guinea-pigs (400–500 g) were used for study of lung function. Male New Zealand White rabbits (2.5–3.5 kg) were used for raising antibodies.

Active sensitization of guinea-pigs to ovalbumin

Guinea-pigs received cyclophosphamide (100 mg kg⁻¹ i.p.) 1 day before sensitization. Animals were sensitized by i.p. injection of 1 ml of an emulsion of Al(OH)₃ (2 mg), pertussis vaccine (0.25 ml) and 1 or 10 µg of ovalbumin (OA), with supplementary injections of such emulsion 3 and 6 weeks later. After 7 weeks, OA (10 µl per site) was injected intradermally and skin thickness was measured 1 h later. A doubling of skin thickness indicated sensitization. Animals which did not respond to intradermal OA were not used.

¹ Author for correspondence at present address: Glaxo Group Research Ltd., Park Road, Ware, Hertfordshire, SG12 0DP.

² Present address: First Department of Internal Medicine, Gunma University School of Medicine, 3-39-15 Showa-machi, Maebashi 371, Japan.

³ Present address: Royal College of Surgeons, Department of Pharmacology, Lincoln's Inn Fields, London WC2A 3PN.

Antigen exposure

Sensitized guinea-pigs were placed in a 16 litre plexiglass chamber which was partitioned into 4 equal sections. Each section housed a guinea-pig, which was restrained by the neck. The snout of each animal was placed in a nose-cone attached to the outlet of a DeVilbiss nebuliser. Animals were exposed for one hour to an aerosol of OA (10 ml of 0.1% solution) which was generated in the nebuliser using compressed air (71 min^{-1}). This procedure does not lead to an anaphylactic response. At various times after exposure, airway reactivity to intravenous spasmogens was measured in anaesthetized animals and lungs were subsequently lavaged. Guinea-pigs sensitized with $1 \mu\text{g}$ of OA were tested at 24, 48, 72 h and 7 days after exposure to OA, whereas guinea-pigs sensitized with $10 \mu\text{g}$ were tested additionally at 4, 8 and 12 h after exposure to OA. Control groups included sensitized guinea-pigs not exposed to allergen and naïve guinea-pigs exposed to OA and tested 24, 48 or 72 h after exposure.

Measurement of lung function and airway reactivity to histamine and $\text{PGF}_{2\alpha}$

Guinea-pigs were anaesthetized with sodium phenobarbitone (100 mg kg^{-1} i.p.) and sodium pentobarbitone (30 mg kg^{-1} i.p.) and paralysed with gallamine (10 mg kg^{-1} i.m.). Animals were ventilated (8 ml kg^{-1} , 1 Hz) via a tracheal cannula with a mixture of air and oxygen (1:1 v/v). Air flow was monitored by a pneumotachograph (type 0000, Fleisch, Switzerland) in line with the respiratory pump). Pressure changes within the pneumotachograph were measured with a differential pressure transducer (MP45-871, Validyne, U.S.A.). Pressure changes within the thorax were monitored directly via an intrathoracic cannula, and the differential pressure between the trachea and thorax was measured with a differential pressure transducer (PM45-24, Validyne, U.S.A.). From these measurements of flow and differential pressure, both airway resistance (R_L) and compliance (C_{dyn}) were calculated breath by breath with a respiratory analyser (Model 6, Buxco, U.S.A.). Preparations were allowed to stabilize for 10 to 15 min, after which the lungs were hyperinflated for 3–5 respiratory cycles by increasing the respiratory pump output to 16 ml kg^{-1} . After this manoeuvre, values for basal R_L and C_{dyn} were recorded. To assess airway reactivity, spasmogens (histamine, 1.0, $1.8 \mu\text{g kg}^{-1}$ and $\text{PGF}_{2\alpha}$ $56 \mu\text{g kg}^{-1}$) were administered intravenously at 10 min intervals via a cannulated jugular vein.

Bronchoalveolar lavage and cell counting

After measurement of lung function parameters, guinea-pigs were killed with sodium pentobarbitone (100 mg kg^{-1} i.v.) and lungs were lavaged with $6 \times 10 \text{ ml}$ aliquots of Ca^{2+} and Mg^{2+} free Hank's balanced salt solution (HBSS), containing bovine serum albumin (BSA, 0.3%), EDTA (10 mM) and HEPES (10 mM). Lavage fluid was centrifuged (200 g for 10 min) and the cell pellet resuspended in 1 ml of supplemented HBSS. Twenty μl of the cell suspension was added to 180 μl of Turk's solution (1:20 dilution) and total cells were counted in an haemocytometer. Differential cell counts were made from smears stained by Leishman's stain. Cells were identified and counted under oil immersion ($\times 1000$), approximately 500 cells per smear were counted and the total population of each cell type was calculated.

Drug administration

Drugs were administered for seven days (6 days before antigen exposure) via an osmotic mini-pump (Alzet), implanted subcutaneously in the nuchal region under ether anaesthesia. Animals receiving saline or a solvent mixture (polyethylene glycol and ethanol, 30/70 v/v) acted as controls. AH 21-132,

dexamethasone, ketotifen and mepyramine were administered at $1 \text{ mg kg}^{-1} \text{ day}^{-1}$. Aminophylline was administered at $10 \text{ mg kg}^{-1} \text{ day}^{-1}$ and salbutamol at 1.0 or $0.1 \text{ mg kg}^{-1} \text{ day}^{-1}$.

Passive cutaneous anaphylaxis

Blood (5 ml) was removed by cardiac puncture from a group of sensitized ($10 \mu\text{g}$ OA) guinea-pigs ($n = 30$) under ether anaesthesia. Serum was collected 24 h after clot retraction, pooled and centrifuged (400 g for 10 min). Aliquots of serum were stored at -20°C . Naïve guinea-pigs were shaved on both flanks and one hour later, marked skin sites were injected with diluted (1:100) serum from hyperimmune ($10 \mu\text{g}$ OA) or normal animals. Three days later, other sites were injected with diluted (1:100) hyperimmune serum. After a further 3 days, Evans blue dye (2.5% w/v in normal saline) and ^{125}I -human serum albumin ($1 \mu\text{Ci}$ per animal), in a final volume of 0.5 ml, were injected intravenously. Within 10 min, sensitized and non-sensitized skin sites were injected with OA ($10 \mu\text{g}$ per site). Thirty min later, guinea-pigs were killed by cervical dislocation and 1 ml of blood was withdrawn by cardiac puncture. The injection sites were removed with a wad-punch (15 mm diameter) and the radioactive content of blood and skin sites was determined in an automatic gamma counter (LKB). Plasma protein extravasation was expressed as μl whole blood equivalent (Williams & Morley, 1973). This procedure allowed measurement of the ability of serum from sensitized guinea-pigs to sensitize skin of naïve guinea-pigs after 3 or 6 days.

Preparation of antibodies to guinea-pig platelets and neutrophils

Preparation of lytic antibodies to guinea-pig platelets or neutrophils has been described in detail elsewhere (Sanjar *et al.*, 1990; Hutson *et al.*, 1989). Briefly, purified platelets (from blood, ca 5×10^8) or neutrophils (from peritoneal exudates, ca 5×10^7) from the guinea-pig were emulsified with Freund's complete adjuvant (1:1) and injected subcutaneously into the neck and flank of rabbits. This procedure was repeated on two further occasions at three-weekly intervals after which rabbits were anaesthetized (sodium pentothal, $30\text{--}50 \text{ mg kg}^{-1}$ i.v.) and bled by cardiac puncture. Serum was collected 24 h later, heat inactivated (56°C for 30 min) and absorbed with guinea-pig erythrocytes. After centrifugation (2000 g for 10 min.), sera were divided into aliquots and stored at -20°C .

Materials

The following materials have been used: aluminium hydroxide (Sandoz), bovine gamma globulin (Miles), bovine serum albumin (Fluka), cyclophosphamide (Sigma), EDTA (Merck), ether (Siegfried), Evans blue dye (Merck), Freund's complete adjuvant (Difco), gallamine (Davis & Geck), Hanks balanced salt solution (Gibco), HEPES (Gibco), histamine (Fluka), ^{125}I -human serum albumin (Amersham), Leishmans stain (Merck), ovalbumin (Fluka), percoll (Pharmacia), pertussis vaccine (Swiss serum & vaccine Institute), prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$, Sigma), sodium pentobarbitone (Merck), sodium phenobarbitone (Merck), sodium pentothal (Merck), Turks solution (Merck), AH 21-132, *cis*-6-(*p*-acetoamidophenyl)-1,2,3,4,10b-hexahydro-8,9-demethoxy-2-methyl (benzo-[c][1,6]-naphthyridine, Sandoz), ketotifen, mepyramine (Sandoz), aminophylline (Siegfried), dexamethasone (Sigma), ethanol, polyethylene glycol, tartaric acid and ascorbic acid (Merck) and Tris buffer (Fluka).

Statistical comparison

Data are presented as means $\pm$ s.e.mean and Student's *t* test for groups of unequal size was used for comparison between treatments.

Results

Comparison of naïve and sensitized guinea-pigs

Only sensitized guinea-pigs in which there was a substantial increased skin thickness ($>100\%$) following intradermal injection of OA ($10\text{ }\mu\text{g}$ per site) were used in this study. Serum from these animals could sensitize the skin of naïve guinea-pigs for up to 6 days. Intradermal injection of OA ($10\text{ }\mu\text{g}$ per site) into non-sensitized sites induced an increased plasma protein extravasation (IPPE) of $14 \pm 1\text{ }\mu\text{l}$ whole blood equivalent, whereas injection at sites sensitized 3 or 6 days previously, induced IPPE of 148 ± 7 and $66 \pm 12\text{ }\mu\text{l}$ whole blood equivalent, respectively ($n = 8$). These observations indicate that responses mediated by IgE-type antibodies contribute substantially to reactions in actively sensitized animals.

Basal airway function (R_L and C_{dyn}), airway reactivity to histamine ($1.0\text{--}1.8\text{ }\mu\text{g kg}^{-1}$) or $\text{PGF}_{2\alpha}$ ($56\text{ }\mu\text{g kg}^{-1}$) and cellular content of bronchoalveolar lavage (BAL) fluid were determined in naïve guinea-pigs and guinea-pigs sensitized with either $1\text{ }\mu\text{g}$ or $10\text{ }\mu\text{g}$ of OA. Sensitized animals did not differ from naïve animals in any of the lung function parameters measured (Table 1). However, sensitized guinea-pigs exhibited a small, but significant, increase in the total eosinophil numbers recovered in BAL (Table 1), and in the incidence of

eosinophils, when compared as a percentage of total cells (naïve, $5.0 \pm 0.7\%$; $1\text{ }\mu\text{g}$ OA, $10.6 \pm 2.0\%$; $10\text{ }\mu\text{g}$ OA, $16.5 \pm 3.0\%$).

Exposure of naïve guinea-pigs to ovalbumin

Naïve guinea-pigs exposed to an aerosol of OA (0.1%) exhibited minimal changes in basal lung function or airway reactivity to histamine ($1.8\text{ }\mu\text{g kg}^{-1}$) or $\text{PGF}_{2\alpha}$ ($56\text{ }\mu\text{g kg}^{-1}$) (Table 2). There was a significant increase in total cell numbers recovered in BAL at 24 ($46 \pm 7 \times 10^6$, $P < 0.001$, $n = 5$) and 48 ($31 \pm 3 \times 10^6$, $P < 0.024$, $n = 5$) hours after exposure to OA when compared with naïve guinea-pigs not exposed to OA ($21 \pm 3 \times 10^6$, $n = 5$) (Table 2).

Exposure of sensitized guinea-pigs to ovalbumin

Animals sensitized with $1\text{ }\mu\text{g}$ ovalbumin Exposure of this group of guinea-pigs to an aerosol of OA (0.1%) for 1 h did not induce overt respiratory distress. Basal lung function (R_L and C_{dyn}) and airway reactivity to histamine ($1.8\text{ }\mu\text{g kg}^{-1}$) or $\text{PGF}_{2\alpha}$ ($56\text{ }\mu\text{g kg}^{-1}$) remained unchanged at all time points (24, 48, 72 h and 1 week after challenge), when compared with sensitized guinea-pigs not exposed to OA (0.1%) (Table 3). However, there was an increase in all cell types in BAL fluid,

Table 1 Comparison of airway responses and bronchial cell populations in naïve and sensitized guinea-pigs

			Airway responses						
Sensitization (μg ovalbumin)	R_L	C_{Dyn}	Histamine		$\text{PGF}_{2\alpha}$	Macro	Cell populations ($\times 10^6$)		
			(1.0)	(1.8)			Eosin	Neut	Lymph
None	130 ± 4	1.0 ± 0.1	27 ± 4	140 ± 20	120 ± 26	17 ± 2	1 ± 0.2	0.5 ± 0.1	2 ± 0.5
1	133 ± 4	1.3 ± 0.1	27 ± 8	131 ± 40	114 ± 36	20 ± 2	$3 \pm 1^*$	0.5 ± 0.1	4 ± 1
10	145 ± 4	1.0 ± 0.1	32 ± 4	166 ± 33	138 ± 33	16 ± 1	4 ± 1	0.4 ± 0.1	2 ± 0.4

R_L = airway resistance ($\text{cmH}_2\text{O l}^{-1} \text{s}^{-1}$), C_{dyn} = dynamic compliance ($\text{ml cmH}_2\text{O}^{-1}$). Histamine and prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) indicate increases in R_L above basal values when histamine (1 or $1.8\text{ }\mu\text{g kg}^{-1}$) or $\text{PGF}_{2\alpha}$ ($56\text{ }\mu\text{g kg}^{-1}$) was injected intravenously. Macro = macrophages; Eosin = eosinophils; Neut = neutrophils; Lymph = lymphocytes. Results are expressed as mean $\pm$ s.e.mean (obtained from at least 10 guinea-pigs). Student's t test was used to assess statistical significance of differences and * indicates a statistical significance, $P < 0.025$.

Table 2 Airway responses and bronchial cell populations after exposure of naïve guinea-pigs to an aerosol of ovalbumin (0.1%)

Time	R_L	C_{dyn}	Airway responses		$PGF_{2\alpha}$	Macro	Cell populations ($\times 10^6$)		
			Histamine				Eosin	Neut	Lymph
			(1.0)	(1.8)					
0	130 ± 4	1.0 ± 0.1	27 ± 4	140 ± 20	120 ± 26	17 ± 2	1 ± 0.2	0.5 ± 0.1	2 ± 0.5
24	119 ± 6	1.7 ± 0.3	39 ± 6	86 ± 12	132 ± 58	28 ± 6	3 ± 2	2 ± 1	$5 \pm 1^*$
48	124 ± 8	1.6 ± 0.3	19 ± 3	112 ± 26	206 ± 112	24 ± 2	3 ± 1	$1 \pm 0.2^*$	$4 \pm 1^*$
72	ND	ND	ND	ND	ND	16 ± 2	1 ± 0.3	$1 \pm 0.3^*$	3 ± 1
1 week	ND	ND	ND	ND	ND	15 ± 2	2 ± 0.2	0.5 ± 0.2	1 ± 0.2

R_L = airway resistance ($\text{cmH}_2\text{O l}^{-1} \text{s}^{-1}$), C_{dyn} = dynamic compliance ($\text{ml cmH}_2\text{O}^{-1}$). Histamine and $\text{PGF}_{2\alpha}$ indicate increases in R_L above basal values when histamine (1.0 or $1.8\text{ }\mu\text{g kg}^{-1}$) or $\text{PGF}_{2\alpha}$ ($56\text{ }\mu\text{g kg}^{-1}$) was injected intravenously. Macro = macrophages; Eosin = eosinophils; Neut = neutrophils; Lymph = lymphocytes; ND = not done. Each time point comprises 5 guinea-pigs and results are expressed as mean $\pm$ s.e.mean. Student's t test was used to assess statistical significance of differences and * indicates a statistical significance, $P < 0.025$.

Table 3 Airway responses and bronchial cell populations after exposure of sensitized guinea-pigs ($1\text{ }\mu\text{g}$) to an aerosol of ovalbumin (0.1%)

Time	R_L	C_{dyn}	Airway responses		$PGF_{2\alpha}$	Macro	Cell populations ($\times 10^6$)		
			Histamine				Eosin	Neut	Lymph
			(1.0)	(1.8)					
0	133 $\pm$ 4	1.3 $\pm$ 0.1	27 $\pm$ 8	131 $\pm$ 40	114 $\pm$ 36	20 $\pm$ 2	3 $\pm$ 1	0.5 $\pm$ 0.1	4 $\pm$ 1
24	123 $\pm$ 4	1.3 $\pm$ 0.1	28 $\pm$ 7	146 $\pm$ 37	89 $\pm$ 37	31 $\pm$ 4*	20 $\pm$ 3*	6 $\pm$ 1*	4 $\pm$ 1
48	136 $\pm$ 7	1.0 $\pm$ 0.1	48 $\pm$ 13	187 $\pm$ 44	524 $\pm$ 196	24 $\pm$ 3	20 $\pm$ 3*	6 $\pm$ 1*	5 $\pm$ 1
72	126 $\pm$ 12	1.2 $\pm$ 0.2	28 $\pm$ 6	167 $\pm$ 39	107 $\pm$ 21	23 $\pm$ 2	17 $\pm$ 3*	3 $\pm$ 1*	4 $\pm$ 0.5
1 week	116 $\pm$ 4	0.9 $\pm$ 0.2	42 $\pm$ 21	206 $\pm$ 184	ND	16 $\pm$ 3	11 $\pm$ 3*	0.2 $\pm$ 0.1	2 $\pm$ 1

R_L = airway resistance ($\text{cmH}_2\text{O l}^{-1} \text{s}^{-1}$), C_{dyn} = dynamic compliance ($\text{ml cmH}_2\text{O}^{-1}$). Histamine and $\text{PGF}_{2\alpha}$ indicate increases in R_L above basal values when histamine (1.0 or $1.8\text{ }\mu\text{g kg}^{-1}$) or $\text{PGF}_{2\alpha}$ ($56\text{ }\mu\text{g kg}^{-1}$) was injected intravenously. Macro = macrophages; Eosin = eosinophils; Neut = neutrophils; Lymph = lymphocytes; ND = not done. Each time point comprises 10 guinea-pigs except the $\text{PGF}_{2\alpha}$ column, where 5 animals received the spasmogen. Results are expressed as mean $\pm$ s.e.mean. Student's t test was used to assess statistical significance of differences and * indicates a statistical significance, $P < 0.025$.

Table 4 Airway responses and bronchial cell populations after exposure of sensitized guinea-pigs (10 µg) to an aerosol of ovalbumin (0.1%)

Time	R_L	C_{dyn}	Airway responses		$PGF_{2\alpha}$	Macro	Cell populations ($\times 10^6$)		
			Histamine				Eosin	Neut	Lymph
			(1.0)	(1.8)					
0	145 ± 4	1.0 ± 0.1	32 ± 4	166 ± 33	138 ± 33	16 ± 1	3 ± 1	0.4 ± 0.1	2 ± 0.4
4	183 ± 12*	0.8 ± 0.2	77 ± 15*	267 ± 49	392 ± 102*	19 ± 3*	5 ± 1	16 ± 3*	3 ± 0.4
8	170 ± 5*	0.8 ± 0.1	68 ± 16	463 ± 70*	339 ± 85*	35 ± 3*	16 ± 3*	17 ± 3*	5 ± 1*
12	165 ± 8*	0.7 ± 0.1	92 ± 25*	405 ± 72*	412 ± 109*	35 ± 3*	29 ± 4*	23 ± 5*	4 ± 1*
24	140 ± 5	1.1 ± 0.1	76 ± 14*	451 ± 116*	322 ± 111	34 ± 3*	33 ± 4*	14 ± 3*	7 ± 1*
48	145 ± 8	1.2 ± 0.2	60 ± 12	337 ± 58*	233 ± 61	33 ± 5*	30 ± 6*	10 ± 3*	5 ± 1*
72	134 ± 3	1.3 ± 0.1	52 ± 9	248 ± 45	221 ± 68	28 ± 3*	21 ± 2*	4 ± 1*	3 ± 1
1 week	136 ± 7	1.6 ± 0.2	34 ± 7	202 ± 24	260 ± 100	22 ± 2	14 ± 1*	0.4 ± 0.1	2 ± 0.3

R_L = airway resistance ($\text{cmH}_2\text{O l}^{-1} \text{s}^{-1}$), C_{dyn} = dynamic compliance ($\text{ml cmH}_2\text{O}^{-1}$). Histamine and $PGF_{2\alpha}$ indicate increases in R_L above basal values when histamine (1.0 or $1.8 \mu\text{g kg}^{-1}$) or $PGF_{2\alpha}$ ($56 \mu\text{g kg}^{-1}$) were injected intravenously. Macro = macrophages; Eosin = eosinophils; Neut = neutrophils; Lymph = lymphocytes. Each time point comprises 10 guinea-pigs and results are expressed as mean $\pm$ s.e.mean. Student's *t* test was used to assess statistical significance of differences and * indicates a statistical significance, $P < 0.025$.

except lymphocytes (Table 3). The largest increase was observed in the eosinophil population, which comprised $33 \pm 5\%$ of total cells 24 h after exposure to OA, as compared with $11 \pm 2\%$ in sensitized animals not exposed to OA. The proportion of eosinophils in BAL fluid remained significantly elevated for up to one week after exposure. Epithelial cells were also observed in BAL fluid at all time points following antigen exposure; the incidence of detached epithelial cells was increased but these changes could not be quantified as these cells were usually found clumped together.

Animals sensitized with 10 µg ovalbumin Exposure of these animals to an aerosol of OA (0.1%) did not provoke obvious respiratory distress. However, in anaesthetized guinea-pigs, basal R_L was significantly higher between 4 and 12 h after exposure to OA, although C_{dyn} was unaffected (Table 4). Intravenous injection of histamine ($1.8 \mu\text{g kg}^{-1}$) or $PGF_{2\alpha}$ ($56 \mu\text{g kg}^{-1}$) revealed increased reactivity to both spasmogens (Table 4). Airway reactivity to histamine was significantly greater at 8, 12, 24 and 48 h. Airway reactivity to $PGF_{2\alpha}$ was significantly greater at 4, 8 and 12 h; reactivity to $PGF_{2\alpha}$ was increased at all time points after exposure to OA, but the difference did not achieve significance at 24, 48, 72 h and 1 week because of considerable inter-animal variation. Guinea-pigs exposed to OA exhibited a prompt accumulation of cells into the pulmonary airway lumen, such that 4 h after exposure to antigen, total cell numbers in BAL fluid had increased to $42.3 \pm 3.1 \times 10^6$ and reached a maximum of $93 \pm 8.3 \times 10^6$ by 12 h; for comparison, $22.7 \pm 1.9 \times 10^6$ cells were recovered from sensitized guinea-pigs not exposed to OA. Neutrophilia was most pronounced between 4 and 12 h, with a steady decline thereafter (Table 4). Eosinophil accumulation into the airway lumen had a slower onset, but was more protracted than neutrophil accumulation, with a significant increase persisting for at least one week (Table 4). Expression of eosinophil counts as percentage of the total cell population, revealed the predominance of this cell type, since eosinophils comprised approximately 38% of the cells recovered in BAL fluid one week after exposure to OA. A temporal relationship between airway hyperreactivity to histamine and pulmonary airway eosinophilia was apparent (Figure 1). However, when airway reactivity to histamine was compared with airway eosinophilia for each animal between 4 h and 7 days following exposure to OA, no correlation was evident (Figure 2). There was also no correlation between airway reactivity and accumulation of any other cell type in BAL fluid.

Drug effects on airway hyperreactivity

None of the drugs tested in this study diminished airway hyperreactivity as indicated by the reaction to $PGF_{2\alpha}$. Ketotifen and mepyramine fully inhibited responses to histamine,

Figure 1 Exposure of sensitized guinea-pigs (10 µg ovalbumin) to an aerosol of ovalbumin (0.1%) induces pulmonary airway eosinophil accumulation (■) and increased airway reactivity to histamine ($1.8 \mu\text{g kg}^{-1}$) (●). Bars indicate s.e.mean and *denotes $P < 0.025$ (Student's *t* test). R_L = airway resistance.

Figure 2 Absence of correlation ($r = 0.18$) between antigen-induced pulmonary airway eosinophil accumulation and airway hyperreactivity (measured as increase in airway resistance R_L to histamine ($1.8 \mu\text{g kg}^{-1}$) in individual sensitized guinea-pigs (10 µg ovalbumin) at various time points (4 h to 7 days) following inhalation of antigen (ovalbumin 0.1%).

Table 5 Effect of anti-asthma drugs upon antigen-induced airway hyperreactivity and eosinophilia in sensitized guinea-pigs (10 µg ovalbumin) 24 h after challenge

Treatment	n	Airway reactivity to Histamine		PGF _{2α}	Cells (× 10 ⁶)	
		(1.0)	(1.8)		Neutrophil	Eosinophil
No challenge	10	32 ± 4	166 ± 33	138 ± 33	0.4 ± 0.1	3.8 ± 0.6
Solvent	20	128 ± 30	430 ± 57	502 ± 71	17.8 ± 3.0	32.4 ± 3.2
AH 21-132	10	87 ± 12	453 ± 50	446 ± 123	9.7 ± 1.7*	18.0 ± 1.7*
Aminophylline	10	117 ± 20	471 ± 65	470 ± 108	10.4 ± 2.4	16.6 ± 2.2*
Dexamethasone	10	113 ± 32	496 ± 122	433 ± 136	7.5 ± 1.7*	14.0 ± 3.0*
Ketotifen	10	0	0	328 ± 71	8.3 ± 1.6*	14.5 ± 2.5*
Mepyramine	5	0	0	397 ± 132	6.6 ± 1.7*	24.0 ± 2.0
Salbutamol	4	109 ± 30	457 ± 130	446 ± 180	33.8 ± 8.8	36.8 ± 11.9

Drugs were given at 1 mg kg⁻¹ day⁻¹ except aminophylline (10 mg kg⁻¹ day⁻¹) and salbutamol (0.1 mg kg⁻¹ day⁻¹). Histamine (1.0 or 1.8 µg kg⁻¹) and PGF_{2α} (56 µg kg⁻¹) were injected intravenously and an increased airway resistance (cmH₂O l⁻¹ s⁻¹) above basal value was measured. Results are expressed as mean ± s.e.mean. Student's *t* test was used to assess statistical significance of differences and *indicates a statistical significance, *P* < 0.025.

which accords with their potency as histamine (H₁) antagonists (Table 5).

Drug effects on cellular content of BAL fluid

AH 21-132, aminophylline, dexamethasone and ketotifen significantly (*P* < 0.025) diminished eosinophilia in BAL 24 h after exposure to OA by 44%, 48%, 56% and 55% respectively (Table 5). Although mepyramine effected a small inhibition (25%), this did not achieve statistical significance. All guinea-pigs treated with salbutamol (1 mg kg⁻¹ day⁻¹) died shortly after exposure to antigen. When a lower dose of salbutamol (0.1 mg kg⁻¹ day⁻¹) was used, only one out of five animals died after exposure to OA. No inhibition of eosinophils or other cell types was observed in the BAL fluid of guinea-pigs that had been treated with salbutamol (Table 5). Interestingly, with addition of mepyramine, all drugs which inhibited eosinophil accumulation, also inhibited neutrophil accumulation (Table 5). Total cell numbers in BAL 24 h after exposure to OA (106.7 ± 9 × 10⁶) were not significantly reduced by any of the drugs tested except for dexamethasone (66.7 ± 7 × 10⁶, *P* < 0.005); conversely, animals treated with salbutamol showed a significant increase of total cell numbers (154.8 ± 8.14 × 10⁶, *P* < 0.025) recovered in BAL fluid.

Effect of depletion of platelets or neutrophils

Intravenous injection of anti-platelet (APS) or anti-neutrophil (ANS) antiserum caused selective depletion of circulating platelets and neutrophils respectively without affecting resident cells in BAL fluid. Maximal depletion of each cell

type was observed at 24 h and, whereas platelets returned to the circulation after a further 24 h, neutrophil depletion persisted for 48 h. Experiments with naïve animals revealed that platelet numbers decreased from 508,000 ± 27,859 to 61,500 ± 12,286 µl⁻¹ 24 h after injection of APS (*n* = 10) and neutrophils decreased from 2,858 ± 273 to 166 ± 83 µl⁻¹ 24 h after injection of ANS (*n* = 10). Guinea-pigs treated with APS or ANS were exposed to an aerosol of OA (0.1%) at 24 h, the time point of maximal depletion. Basal cell numbers in BAL fluid were not affected by these treatments and neither pulmonary airway eosinophilia nor airway hyperreactivity were diminished in thrombocytopenic or neutropenic guinea-pigs compared with untreated animals (Figure 3).

Discussion

Bronchial asthma is commonly characterised by increased airway reactivity to spasmogens (Cockcroft, 1983) and eosinophilia of sputum (Epstein, 1972), peripheral blood (Horn *et al.*, 1975) or bronchial tissue (Huber & Koessler, 1922; Dunnill, 1960; Filley *et al.*, 1982). Clinical studies strongly suggest a close link between airway eosinophilia and increased airway reactivity. During reactions to inhaled allergen, an eosinophil chemotactic factor has been detected in serum (Metzger *et al.*, 1986; Hakansson *et al.*, 1989), which accords with bronchial eosinophil accumulation found in patients exhibiting a late asthmatic response (De Monchy *et al.*, 1985; Metzger *et al.*, 1987) who are known to develop airway hyperreactivity (Durham & Kay, 1985; Diaz *et al.*, 1989). It has been proposed that eosinophils cause epithelial damage by releasing major basic protein (Filley *et al.*, 1982; Frigas & Gleich, 1986) which may lead to increased airway reactivity, either by exposing sensory nerve endings (Barnes, 1986) or by removing the protective effects of an epithelial derived relaxant factor (Flavahan *et al.*, 1988).

Other clinical studies indicate that eosinophilia and increased airway reactivity may be unrelated. Thus, increased airway reactivity is observed shortly after resolution of early airway obstruction following exposure to antigen (Thorpe *et al.*, 1987), when eosinophils are not evident in lavage fluid (De Monchy *et al.*, 1985). Increased airway reactivity occurs in normal subjects after viral infection of the upper respiratory tract (Empey *et al.*, 1976); a condition characterised by bronchial epithelial damage and shedding (Hers & Mulder, 1961; Walsh *et al.*, 1961), which is caused by a direct action of the virus (Negroni & Tyrrel, 1959), without an apparent involvement of eosinophils. Conversely, patients with chronic cough and elevated sputum eosinophils have been shown to exhibit no increased airway reactivity (Gibson *et al.*, 1989).

We have used sensitized guinea-pigs to study the relationship between increased airway reactivity and cellular inflammation of the airways, particularly eosinophil accumulation,

Figure 3 A comparison of airway response to histamine (1.8 µg kg⁻¹ i.v.) (a), eosinophil numbers in bronchoalveolar lavage (BAL) fluid (b) and neutrophil numbers in BAL fluid (c) in (A) sensitized guinea-pigs (10 µg ovalbumin) not challenged; (B) sensitized guinea-pigs 24 h after challenge; (C) thrombocytopenic sensitized guinea-pigs 24 h after challenge and (D) neutropenic sensitized guinea-pigs 24 h after challenge. Bars indicate s.e.mean and * denotes *P* < 0.025 (Student's *t* test).

following antigen challenge. The guinea-pig is well suited for such studies since airway hyperreactivity and eosinophilia can readily be demonstrated in this species (Kallos & Kallos, 1984; Dunn *et al.*, 1988; Hutson *et al.*, 1988a; Aoki *et al.*, 1988; Daffonchio *et al.*, 1989). Exposure of sensitized guinea-pigs to aerosols containing a small dose of allergen induced an allergic reaction without anaphylactic bronchospasm. These guinea-pigs invariably developed a pronounced pulmonary airway eosinophilia which persisted for at least one week. Preliminary histological studies indicated that there was a good correlation between bronchial tissue eosinophilia and eosinophils collected in BAL fluid, in agreement with earlier studies (Snella *et al.*, 1987; Dunn *et al.*, 1988). Accordingly, we have chosen to assess eosinophil accumulation in BAL fluid rather than by histology, due to the difficulty in evaluating histological specimens of lung tissue from large numbers of guinea-pigs.

In animals sensitized with 10 µg of OA, airway eosinophilia and increased airway reactivity to histamine or PGF_{2α} followed a similar time course, yet there was no correlation between these two events, nor was accumulation of other cell types correlated with airway hyperreactivity. Dissociation between pulmonary airway eosinophil accumulation and increased airway reactivity was most clearly evident in guinea-pigs sensitized with 1 µg of ovalbumin, since these animals exhibited no airway hyperreactivity to spasmogens, despite a 7 fold increase in eosinophil numbers in BAL fluid. Such observations are in agreement with a recent study where endotoxin-induced airway inflammation, consisting of eosinophils and neutrophils, in the guinea-pig was not accompanied by airway hyperreactivity (Folkerts *et al.*, 1988). Furthermore, depletion of leukocytes in guinea-pigs by steroids did not suppress bronchial hyperreactivity induced by ozone (Murlas & Roum, 1985). It is interesting to note that stimuli such as ozone (Fabbri *et al.*, 1984; Holroyde & Norris, 1988) or viral infections, which cause increased airway reactivity in experimental animals or man, are more commonly associated with epithelial damage rather than eosinophil accumulation. Eosinophils may represent only one way of causing such damage, particularly in severe asthma. This proposition is supported by recent biopsy studies from mild or asymptomatic asthma patients (Laitinen *et al.*, 1985; Beasley *et al.*, 1989) in whom epithelial damage is a common finding with little or no eosinophil involvement. Our results indicate that eosinophil accumulation *per se* does not lead to increased airway reactivity, since the group of animals sensitized with 1 µg of OA responded to antigen exposure with a severe bronchial eosinophilia without increased airway reactivity. Eosinophils have been classified as normodense or hypodense on the basis of sedimentation on discontinuous metrizamide or Percoll gradients (Winqvist *et al.*, 1982). Hypodense eosinophils exhibit greater oxygen consumption (Winqvist *et al.*, 1982), are more reactive to a variety of stimuli, thus generating greater quantities of leukotriene C₄ (LTC₄), and kill parasite larvae more actively (Rothenberg *et al.*, 1988). Although the state of activation of cells obtained from BAL was not determined, it is possible that the group of animals which became hyperreactive (sensitized with 10 µg of OA) had more activated or hypodense eosinophils. However, on the basis of studies in patients (Winqvist *et al.*, 1982; Prin *et al.*, 1984), it is not unreasonable to assume that all eosinophils found in BAL were activated to a degree, since they had to cross several body compartments in order to arrive in the airway lumen.

Distinction between pulmonary airway eosinophilia and airway hyperreactivity could be confirmed by use of anti-asthma drugs. Aminophylline, dexamethasone, ketotifen and

AH 21-132 significantly inhibited eosinophil and neutrophil accumulation in BAL without affecting airway hyperreactivity. Our data on inhibition of eosinophil accumulation are in agreement with those in the literature, since prophylactic anti-asthma drugs can inhibit eosinophilia, induced by different stimuli, in guinea-pigs (Murlas & Roum, 1985; Sanjar *et al.*, 1989; Sanjar *et al.*, 1990), rats (Spicer *et al.*, 1985) or man (Ohman *et al.*, 1972; Horn *et al.*, 1975; Gobel, 1979; Diaz *et al.*, 1984). It was surprising that none of the drugs used in this study inhibited airway hyperreactivity. Steroids are known to inhibit increased airway reactivity after antigen challenge (Cockcroft & Murdock, 1987) or exposure to toluene diisocyanate (Fabbri *et al.*, 1985). However, efficacy of compounds such as aminophylline or ketotifen at inhibiting acute increases in airway hyperreactivity is unclear and histamine (H₁) receptor antagonists and β-adrenoceptor agonists (Cockcroft & Murdock, 1987; Lai *et al.*, 1989) are ineffective. Of particular interest was the effect of salbutamol since animals treated with the high dose (1 mg kg⁻¹ day⁻¹) did not survive antigen exposure. Increased mortality has previously been demonstrated in guinea-pigs treated with β-adrenoceptor agonists before exposure to an aerosol of histamine (Conolly *et al.*, 1971) or intravenous antigen (Izard *et al.*, 1971), which may reflect the capacity of these compounds to induce airway hyperreactivity (Sanjar & Morley, 1988). Guinea-pigs treated with a lower dose of salbutamol showed no inhibition of eosinophil accumulation, in agreement with an earlier study in which salbutamol (albuterol) was used (Hutson *et al.*, 1988b). However, fenoterol has been shown to inhibit eosinophil accumulation in airways of passively sensitized guinea-pigs (Fugner, 1989). The differences in the activity of these β-adrenoceptor agonists are not clear and may relate to relative potency or properties other than β-adrenoceptor stimulation. Animals treated with the lower dose of salbutamol showed a significantly greater increase of total cell numbers in BAL fluid 24 h after antigen exposure. This observation could relate to the bronchodilator efficacy of salbutamol which would prevent local airway constriction, thereby allowing greater entry of antigen into the airways. This proposition finds support in a recent clinical study, where the use of a short acting bronchodilator, rimeterol hydrobromide, allowed greater exposure of asthma patients to antigen (Lai *et al.*, 1989), thereby inducing late-onset airway obstruction in subjects who previously gave only an early airway obstruction. On the basis of these observations, the sensitized guinea-pig model differs from allergic asthma principally by the lack of inhibition of airway hyperreactivity by steroids.

Removal of circulating platelets (Pinckard *et al.*, 1977; Butler & Smith, 1981; Lellouch-Tubiana *et al.*, 1988) or neutrophils (Wedmore & Williams, 1981; O'Byrne *et al.*, 1984) has been shown to diminish inflammatory reactions. In the present study, neither platelets nor neutrophils contribute to either antigen-induced airway eosinophilia or hyperreactivity. These observations are in agreement with studies of platelet-activating factor (PAF)-induced pulmonary airway eosinophilia, when neither platelet nor neutrophil depletion influenced the eosinophilic response (Sanjar *et al.*, 1990). Furthermore, antigen-induced late-onset airway obstruction and pulmonary eosinophil accumulation was also not influenced by neutrophil depletion (Hutson *et al.*, 1989).

The present observations indicate that the association between pulmonary airway eosinophilia and acute airway hyperreactivity reflects a common origin (the allergic reaction) rather than a causal relationship, thus airway hyperreactivity and eosinophil accumulation occur simultaneously but independently of each other.

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(Received September 21, 1989

Revised November 22, 1989

Accepted December 8, 1989)