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Role of interleukin-5 and substance P in development of airway hyperreactivity to histamine in guinea-pigs

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ABSTRACT: In this study, we examined the mechanism by which bronchoalveolar lavage (BAL) cells induced hyperreactivity of the trachea *in vitro*. As both interleukin-5 (IL-5) and substance P (SP) appeared to be involved, the effect of these mediators was examined *in vivo*.

Tracheae were incubated with BAL cells from ovalbumin or saline challenged animals, and from naive animals, in the absence or presence of either IL-5, SP, or both. In addition, the effect of intra-airway application of IL-5, SP, both, or vehicle on tracheal hyperreactivity was examined.

Incubation of tracheae with BAL cells from ovalbumin challenged animals induced an increase (30±10%) in the maximal response to histamine. The hyperreactivity could be completely inhibited by co-incubation with the 5-lipoxygenase inhibitor, AA861. The hyperreactivity could be mimicked by incubation of tracheae with BAL cells from naive animals in the presence of IL-5 and SP. After *in vivo* administration of either IL-5 or SP, maximal responses to histamine were increased and amounted to 105±35 and 101±37%, respectively. Administration of IL-5 but not SP induced a significant increase in the number of eosinophils (67±22%) and eosinophil peroxidase (EPO) activity (94±33%) in BAL cells. The simultaneous administration of IL-5 and SP did not potentiate the hyperreactivity and eosinophilia observed with IL-5 alone.

These data suggest that IL-5 is important in the recruitment of eosinophils, whereas both IL-5 and substance P are involved in the induction of airway hyperreactivity.

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Airway hyperreactivity to bronchoconstrictor mediators is a main characteristic in a majority of asthmatic patients and correlates well with the severity of the disease [1]. Airway hyperreactivity often coincides with the infiltration of inflammatory cells, in particular eosinophilic granulocytes [2]. The mechanistic relationship between hyperreactivity and cell infiltration is currently unknown.

Recently, we and others demonstrated that antibodies to interleukin-5 (IL-5) inhibit the airway hyperreactivity and eosinophil infiltration in a guinea-pig model of allergic asthma [3, 4]. Conversely, administration of IL-5 has been demonstrated to induce airway eosinophilia and hyperreactivity in guinea-pigs and mice [3, 5, 6]. These data underline the putative role of the eosinophil in airway hyperreactivity. On the other hand, in guinea-pigs depleted of sensory neuropeptides by capsaicin treatment, ovalbumin challenge does not induce airway hyperreactivity despite the infiltration of eosinophils [7, 8]. A possible explanation is that as well as other factors IL-5 is involved in the recruitment of eosinophils, whereas sensory neuropeptides seem to be involved in the process by which eosinophils induce hyperreactivity. In agreement with this, IL-5 enhances adhesion of eosinophils to endothelial cells and is an eosinophil chemoattractant [9, 10]. Furthermore, it is well-known that IL-5 primes eosinophils for enhanced effector function to activators [11–15]. Although substance P (SP) may also act as a priming substance for eosinophil chemoattractants [16], little is known about the effect on eosinophil activation.

To investigate the role of inflammatory cells in the induction of airway hyperreactivity, BAL cells from ovalbumin-challenged guinea-pigs were incubated with isolated tracheal rings from naive animals. Furthermore, the effect of IL-5 or SP, or the combination of these factors, on the responsiveness of guinea-pig trachea was examined after incubation *in vitro* or after intra-airway application *in vivo*.

Materials and methods

Animal sensitization and challenge

The animals used in this study were specified pathogen-free male Dunkin Hartley guinea-pigs, weighing 350–500 g (Harlan Porcellus, UK). Water and commercial chow were allowed *ad libitum*. The guinea-pigs were free of respiratory infections as assessed by the health monitoring quality control report by Harlan Porcellus (UK). Animals were sensitized with a single injection of ovalbumin (20 mg·kg^{-1} *i.p.*) and 14 days later exposed daily for eight consecutive days to either saline aerosol for 10 s, or 2% ovalbumin aerosol (maximal 10–20 s) until dyspnoea signs appeared.

Bronchoalveolar lavage

Bronchoalveolar lavages (BAL) were performed in chronically challenged animals 1 day after the last aerosol exposure, or in naive animals. Animals received a lethal dose of pentobarbital sodium (300 mg·kg^{-1} *i.p.*), the trachea was trimmed free of connective tissue and a small incision was made to insert a cannula into the trachea. The lungs were filled *in situ* with 5–10 mL NaCl-ethylenediamine tetra-acetic acid (EDTA) buffer (0.15 M NaCl, 2.6 mM EDTA) using a syringe. Fluid was withdrawn from the lungs after gentle lung massage and collected in a plastic tube on ice. Washings were repeated and the cell suspensions recovered from one animal were pooled until 50 mL fluid was obtained. The cells were sedimented by centrifugation at 400×g for 10 min at 4°C, and were washed twice with RPMI 1640 medium. The cells were stained with Türk solution and counted. Viability was always more than 95%, as determined by trypan blue exclusion. BAL cells from different groups of animals were pooled in RPMI 1640 supplemented with 5% foetal calf serum (FCS) and gentamicin (50 µg·mL^{-1}) at a concentration of 10^6 cells·mL^{-1}, and subsequently incubated with tracheal rings as described in the next section. Pooled BAL cell preparations were analysed morphologically after centrifugation on microscope slides. Air-dried preparations were fixed and stained with Diff-Quick (Merz & Dade A.G., Düdingen, Switzerland). Differential counts (total of 200 cells) were made under oil immersion microscopy. All materials and buffers used were sterile.

Effect of BAL cells on tracheal reactivity

Naive animals received a lethal dose of pentobarbital sodium (300 mg·kg^{-1} *i.p.*). The trachea was removed and trimmed free of connective tissue and blood vessels, and subsequently cut into pieces of two cartilage rings. The tracheal rings were transferred to 6-well microplates (Costar, Cambridge, MA, USA) in 3 mL RPMI 1640 containing 5% FCS and gentamicin (50 µg·mL^{-1}), in the absence or presence of pooled BAL cells (10^6 cells·mL^{-1}) from ovalbumin sensitized guinea-pigs challenged with ovalbumin or saline, or from naive animals, in the absence or presence of either IL-5 (300 ng·mL^{-1}) or SP (10^{-5} M) and phosphoramidon (10^{-5} M), or a combination of these substances. All materials and buffers used were sterile. Three days after incubation in a CO_2-incubator at 37°C, the tracheal rings were mounted in an organ bath filled with Krebs bicarbonate buffer. Krebs was continuously aerated with a 5% CO_2 and 95% O_2 gas mixture. Changes in length were measured by means of an isometric transducer. After equilibration at 2 g tension, a cumulative histamine concentration-response curve was constructed.

In a separate series of experiments, tracheal rings were incubated for 3 days with pooled BAL cells (10^6 cells·mL^{-1}) from ovalbumin sensitized guinea-pigs challenged with ovalbumin or saline, in the absence or presence of the selective 5-lipoxygenase inhibitor, AA861 (10^{-6} M) (2-(12-hydroxydodeca-5,10-diynyl)-3,5,6-trimethyl-1,4-benzoquinone; Wako, Japan), which was added daily.

Intra-airway administration of IL-5 and SP

Guinea-pigs received either 1 µg IL-5, 10 µg SP, both substances simultaneously, or vehicle consisting of saline with 0.1% bovine serum albumin (BSA), in a volume of 300 µL intranasally under short-lasting anaesthesia (Ketalar® 40 mg·kg^{-1} *i.m.*) and Rompun®, 5 mg·kg^{-1} *s.c.* twice on one day (9 a.m. and 4 p.m.). Twenty four hours after the first administration, guinea-pigs received a lethal dose of pentobarbital sodium (300 mg·kg^{-1}). Bronchoalveolar lavage was performed as described above and differential cell counts of every individual animal were made. Samples from the first lavages (5 mL) were centrifugated at 400×g for 10 min at 4°C and stored at -70°C until eosinophil peroxidase activity was deter-mined (described below). The trachea was isolated and perfused in an organ bath as described previously [17]. Briefly, two hooks were inserted through opposite sides of the tracheal wall with the smooth muscle between them. One hook was attached to a fixed point in the organ bath, the other hook was connected to an isome-tric transducer. Tracheal tension was set at an optimal counterweight of 2 g. The inside of the trachea was perfused with Krebs solution at a constant flow rate of 2 mL·min^{-1} with a peristaltic pump. Every 15 min, the Krebs buffer was refreshed on both sides until a stable tone was reached. Thereafter, cumulative concentrations of histamine were applied intraluminally and the contractions registered on a recorder.

BAL cell and fluid eosinophil peroxidase activity

BAL cells from animals treated with IL-5, SP, or both substances were suspended in 1 mL ice-cold 0.05 M Tris-HCl buffer (pH=8) containing 0.1% Triton X-100. BAL cells were exposed to five freeze-thaw cycles and centrifuged at 1,600×g for 5 min at 4°C. The eosinophil peroxidase (EPO) activity in the supernatant was measured according to the method of STRATH *et al.* [18], which is based on the oxidation of o-phenylenediamine

(OPD) by EPO in the presence of H_2O_2. The substrate solution consists of 10 mM OPD in 0.05 M Tris-HCl buffer (pH=8) and 4 mM H_2O_2 (BDH, Poole, UK). Substrate solution (100 µL) was added to BAL cell supernatant samples (50 µL) in duplicate in a 96-well microplate, and incubated at room temperature for 30 min before stopping the reaction by the addition of 50 µL of 4 M sulphuric acid. The absorbance was then measured at 492 nm using a Titertek Multiskan (Flow Labs, Irvine, UK). Blanks were BAL cell supernatant samples (50 µL) incubated with Tris-HCl buffer. BAL fluid samples were measured in the same way. Serial dilutions of horseradish peroxidase (200 ng·mL^{-1}) were used to quantitate the amount of peroxidase in the samples. Results are expressed as nanograms of peroxidase activity.

Materials

Substance P, phosphoramidon, o-phenylenediamine, horseradish peroxidase, bovine serum albumin grade V and ovalbumin grade V were obtained from Sigma Chemical Co. (St. Louis, USA). RPMI 1640 and FCS were obtained from Gibco BRL (Paisley, Scotland). Recombinant IL-5 was purified by P. Graber (Glaxo Institute for Molecular Biology, Geneva, Switzerland) and kindly donated by D. Fattah (Glaxo, Greenford, UK).

Data analysis

Results are expressed as mean±SEM. Concentration response curves are compared using analysis of variance. Student's t-test was used for comparing numbers of different leucocytes in BAL and the maximal responses and pD_2 values (-log EC_{50}) of the concentration response curves. A p-value of less than 0.05 was considered significant.

Results

In vitro *incubations*

After a 3 day incubation of tracheal rings from naive guinea-pigs with BAL cells from sensitized animals chronically exposed to ovalbumin, the maximum response to histamine was significantly ($p<0.05$) increased by 30±10% compared to those incubated with BAL cells from saline-challenged animals (fig. 1). The pD_2 value of the histamine concentration-response curve (CRC) was also decreased ($p<0.05$) in tracheal rings incubated with BAL cells from ovalbumin-challenged animals. Similar incubations during one day did not affect the tracheal responsiveness to histamine.

In a separate series of experiments, incubations were performed in the absence or presence of the selective 5-lipoxygenase inhibitor, AA861. AA861 completely inhibited the increased maximum response to histamine of tracheal rings incubated with BAL cells from ovalbumin-challenged animals (table 1). The distribution of the different leucocytes in the pooled BAL cells from sensitized animals exposed to ovalbumin or saline are presented in table 2. Ovalbumin-challenged animals contained relatively more eosinophils and less mononuclear cells than saline challenged animals.

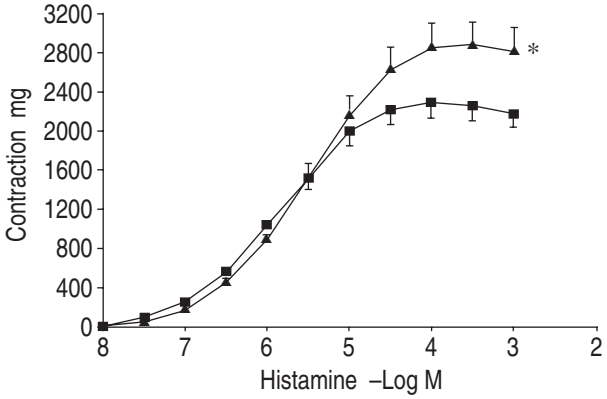

Fig. 1. – Cumulative histamine concentration response curves of tracheal rings (isolated from naive guinea-pigs) which were incubated for 3 days with BAL cells from ovalbumin-sensitized guinea-pigs challenged with ovalbumin (n=18), or saline (n=16). Results are expressed as mean±SEM. *: $p<0.05$, as determined with ANOVA. BAL: bronchoalveolar lavage; ANOVA: analysis of variance. —▲—: ovalbumin; —■—: saline.

Table 1. – Maximal effect (E_{max}) and pD_2 values of histamine concentration response curves of tracheal rings from naive animals incubated with BAL cells from ovalbumin-sensitized animals challenged with ovalbumin or saline in the absence (-) or presence (+) of the selective 5-lipoxygenase inhibitor, AA861 (10^{-6} M)

BAL cells	AA861	E_{max} mg	pD_2	n
Saline	-	2041±283	5.42±0.05	12
Ovalbumin	-	2749±192*	5.35±0.10	10
Saline	+	1838±232	5.56±0.11	12
Ovalbumin	+	1993±210	5.24±0.12	10

Values are presented as mean±SEM. BAL: bronchoalveolar lavage. *: $p<0.05$ compared to all other groups as determined with Student's t-test.

Incubation of tracheal rings from naive guinea-pigs with BAL cells from naive animals for 3 days in the presence of IL-5, SP and phosphoramidon induced a significant ($p<0.05$) increase in the maximum response

Table 2. – Distribution of leucocytes (%) in pooled bronchoalveolar lavage (BAL) cells from ovalbumin-sensitized animals challenged with ovalbumin or saline, or from naive animals

Treatment	Mononuclear cells	Eosinophilic granulocytes	Neutrophilic granulocytes
Data from figure 1 and table 3			
Ovalbumin	40	56	4
Saline	84	14	2
Naive	86	11	3
Data from table 1			
Ovalbumin	58	37	5
Saline	88	11	1

Pooled BAL cells were used for incubation with tracheal rings at a concentration of 10^6 cells·mL^{-1}.

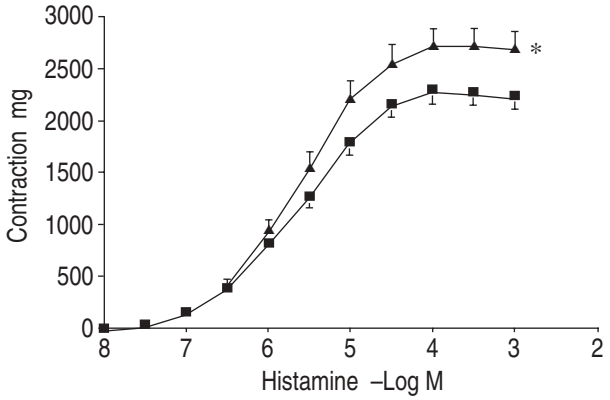

Fig. 2. – Cumulative histamine concentration response curves of tracheal rings (isolated from naive guinea-pigs) which were incubated with (n=22) and without (n=22) interleukin-5 (IL-5, 300 ng·mL^{-1}) substance P (10^{-5} M) and phosphoramidan (10^{-5} M) for 3 days with BAL cells from naive animals. Results are expressed as mean±SEM. *: $p<0.05$, as determined with ANOVA. For abbreviations see legend to figure 1. —■—: control; —▲—: IL-5, substance P and phosphoramidon.

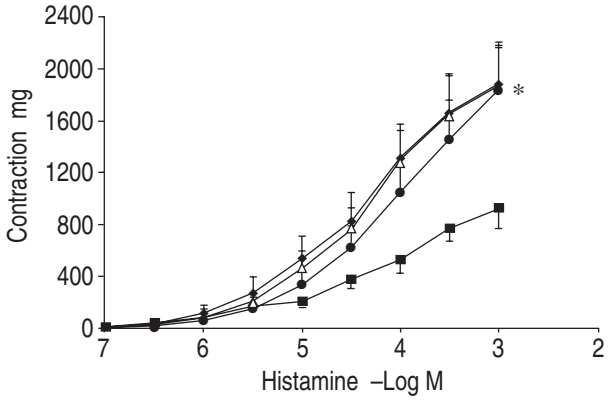

Fig. 3. – Cumulative histamine concentration response curves of perfused tracheal segments isolated from guinea-pigs 1 day after intra-airway application of interleukin-5 (IL-5) (twice 1 µg) (n=4), substance P (SP) (twice 10 µg) (n=6), IL-5 and SP simultaneously (n=5), or vehicle (saline with 0.1% BSA) (n=5) in a volume of 300 µL. Results are expressed as mean±SEM. *: $p<0.01$, as determined with ANOVA. BSA: bovine serum albumen; ANOVA: analysis of variance. —◆—: IL-5 only; —●—: SP only; —Δ—: IL-5 and SP simultaneously; —■—: vehicle.

(20±7%) to histamine compared to those incubated with BAL cells only (fig. 2). The pD_2 values of the histamine CRC did not differ between the two groups. Similar incubations during one day did not change the responsiveness of the trachea (results not shown). The maximal responsiveness and pD_2 values of the histamine CRC of tracheal rings incubated with BAL cells in the presence of either IL-5 or SP and phosphoramidon were not different from those incubated with BAL cells only (table 3). Furthermore, the maximal response and pD_2 value of the histamine CRC of tracheal rings incubated with IL-5, SP and phosphoramidon in the absence of BAL cells did not differ from those incubated without these substances (table 3). The distribution of the different leucocytes in the pooled BAL cells from naive animals are presented in table 2.

Table 3. – Maximal effect (E_{max}) and pD_2 values of histamine concentration response curves of tracheal rings from naive animals incubated with or without BAL cells in the absence (control) or presence of IL-5 (300 ng·mL^{-1}) or SP (10^{-5} M) and phosphoramidon (10^{-5} M), or the combination of these substances

Treatment	E_{max} mg	pD_2	n
With BAL			
Control	2236±115	5.63±0.05	22
IL-5	2164±211	5.61±0.13	13
SP+PM	2223±132	5.66±0.01	22
IL-5+SP+PM	2686±161*	5.62±0.06	22
Without BAL			
Control of IL-5	2153±176	5.52±0.15	4
IL-5	1801±348	5.69±0.29	4
Control of SP	1579±134	5.06±0.13	6
SP+PM	1887±258	5.30±0.14	7
Control of IL-5+SP	1739±246	5.21±0.03	4
IL-5+SP+PM	1643±151	5.24±0.10	4

Values are presented as mean±SEM. IL-5: interleukin-5; SP: substance P; PM: phosphoramidon. For further abbreviations see legend to table 1. *: $p<0.05$ as compared to control (BAL cells only) and determined with Student's t-test.

Intra-airway application of IL-5 and SP

One day after intra-airway administrations of IL-5 (twice 1 µg), the contractions of isolated perfused tracheal segments to cumulative concentrations of histamine were significantly increased (fig. 3). The increases amounted to 150±53, 115±40 and 105±35%, at 10^{-4}, 3×10^{-4} and 10^{-3} M histamine, respectively ($p<0.05$; Student's t-test). Similarly, 1 day after intra-airway administrations of SP (twice 10 µg) the contractions of isolated perfused tracheal segments to cumulative concentrations of histamine were significantly increased (fig. 3). The increases amounted to 90±37 and 101±37% at 3×10^{-4} and 10^{-3} M histamine, respectively ($p<0.05$; Student's t-test). The simultaneous administration of IL-5 and SP induced a similar hyperreactivity of the tracheal segments to histamine as did the administration of either substance alone (fig. 3). The increase of the contractions amounted to 109±41, 150±43, 115±37 and 105±31% at 3×10^{-5}, 10^{-4}, 3×10^{-4} and 10^{-3} M histamine, respectively ($p<0.05$; Student's t-test). The pD_2 values of the histamine concentration response curves did not differ between the experimental groups and vehicle-treated animals (results not shown).

One day after intra-airway administration of IL-5, the number of eosinophils present in BAL was significantly increased by 67±22% ($p<0.05$; Student's t-test) compared to saline-treated animals (table 4). Similarly, the EPO activity of total BAL cells was significantly increased by 94±33% ($p<0.05$, Student's t-test) compared to saline-treated animals (table 5). The numbers of mononuclear cells and neutrophils in BAL were not different between these two groups. Treatment of the guinea-pigs with SP did not induce alterations in the cellular composition of the BAL or change the EPO activity in BAL cells (tables 4 and 5). Animals treated simultaneously with IL-5 and SP showed a similar, although not significant, increase in the number of eosinophils and BAL cell EPO activity to animals treated with IL-5 only (tables 4 and 5).

Table 4. – Numbers of different cell types ($\times 10^5$) in bronchoalveolar lavage fluid of animals treated with IL-5, SP, both or vehicle

Treatment	n	Mononuclear cells	Eosinophilic granulocytes	Neutrophilic granulocytes
Vehicle	6	134±19	18±4	28±10
IL-5	7	141±14	30±4*	50±22
SP	7	151±31	18±5	92±46
IL-5+SP	7	111±19	28±9	55±30

Values are presented as mean±SEM. For abbreviations see legend to table 3. *: $p<0.05$ compared to vehicle treated animals as determined with Student's t-test.

Table 5. – Eosinophil peroxidase (EPO) activity in total bronchoalveolar lavage (BAL) cells, eosinophils and cell-free BAL fluid (BALF)

		Eosinophil peroxidase activity		
		Total BAL cells	Eosinophils	Cell-free BALF
Treatment	n	ng	$ng \cdot 10^{-5}$ cells	$ng \cdot mL^{-1}$
Vehicle	6	937±83	47±7	52±16
IL-5	7	1816±313*	59±6	64±21
SP	7	952±322	67±20	36±5
IL-5+SP	7	1519±488	62±11	60±12

Values are presented as mean±SEM. The amount of EPO activity per eosinophil is obtained by dividing total EPO activity in all BAL cells by the number of eosinophils present in the BAL cells: animals were treated with IL-5, SP, IL-5 and SP, or vehicle For abbreviations see legend to table 3. *: $p<0.05$, compared to vehicle treated animals and determined with Student's t-test.

The number of mononuclear cells and neutrophils was not affected by combined treatment with IL-5 and SP as compared to vehicle-treated animals. The amount of EPO per eosinophil and the EPO activity in cell-free BAL fluid did not differ between the experimental and control groups (table 5).

Discussion

IL-5 and SP have both been implicated in ovalbumin-induced airway hyperreactivity in guinea-pigs [3, 4, 7, 8, 19]. In this study, the effects of IL-5 and SP on the responsiveness of guinea-pig trachea was examined *in vitro* and *in vivo*. It was demonstrated that BAL cells derived from ovalbumin-challenged animals could induce tracheal hyperreactivity *in vitro*, which was dependent on the production of leukotrienes. The tracheal hyperreactivity could be mimicked by incubation of BAL cells from naive animals both with IL-5 and SP. Although only IL-5 induced airway eosinophil infiltration after intra-airway application, either IL-5 or SP induced tracheal hyperreactivity. It might be suggested that IL-5 is important in the recruitment of eosinophils, whereas both IL-5 and substance P are involved in the induction of airway hyperreactivity.

Incubation of tracheal rings with BAL cells from guinea-pigs sensitized and challenged with ovalbumin induced an increase in the maximum response to histamine similar to the hyperreactivity which can be observed after ovalbumin challenge *in vivo* [3]. This suggests that cells present in the BAL of ovalbumin-challenged animals are responsible for this hyperreactivity. Ovalbumin challenge induced a marked increase in the number of eosinophils and neutrophils in BAL [3]. The eosinophilic granulocyte is thought to be involved in the induction of airway hyperreactivity in this animal model. In agreement with this, we recently demonstrated that antibodies to IL-5 inhibited the infiltration of eosinophils and the development of increased tracheal responsiveness in ovalbumin-challenged guinea-pigs [3]. In addition, antibodies to the adhesion molecule, very late activation antigen-4, prevented ovalbumin-induced eosinophil infiltration and hyperreactivity [20].

Evidence exists that neuropeptides are also involved in the induction of airway hyperreactivity but do not play a dominant role in the infiltration of eosinophils [7, 8, 19]. In the present study, we demonstrated that the maximum response of tracheal rings incubated with bronchoalveolar cells from naive animals was increased by co-incubation with IL-5 and SP. Since IL-5 is a cytokine which selectively affects eosinophil differentiation and effector function [11, 13–15] it can be speculated that this cell type is involved in the induction of hyperreactivity. However, we cannot rule out a possible role for other leucocytes present in BAL. Histological examination of tracheal rings after incubation with or without BAL cells in the absence or presence of IL-5 or SP revealed no differences in the epithelial layer and no adhesion of BAL cells to tracheal tissue (unpublished observation). This indicates that a soluble mediator is involved in the induction of hyperreactivity.

It has been shown that purified human or guinea-pig eosinophils, when activated, induce hyperreactivity of guinea-pig trachea to methacholine or histamine [21–23]. The induction of hyperreactivity was mediated by leukotriene C_4 (LTC_4) but not by eosinophil granule proteins [21–23]. The synthesis of LTC_4 after activation of eosinophils has been shown to be largely enhanced by prior incubation with IL-5 [11, 24]. The absence of epithelial damage in our experiments argues against a role for eosinophilic cytotoxic cationic granule proteins in the induction of hyperreactivity [25]. Furthermore, relatively high concentrations are needed for the cytotoxic effects of major basic protein (MBP). However, evidence exists that MBP and other cationic proteins can induce airway hyperreactivity in the absence of epithelial damage [26–28]. In the present study, we demonstrated that the induction of hyperreactivity by BAL cells from ovalbumin-challenged animals could be completely prevented by co-incubation with the 5-lipoxygenase inhibitor, AA861. This observation suggests a role for LTC_4 or other 5-lipoxygenase products in the induction of hyperreactivity by BAL cells.

In contrast to the obligatory presence of IL-5 and SP for the induction of hyperreactivity in isolated trachea, either IL-5 or SP alone induced hyperreactivity after *in*

vivo intra-airway administration. The IL-5-induced hyperreactivity coincided with the infiltration of eosinophils in the BAL fluid. This corroborates the importance of IL-5 in the recruitment of eosinophils. However, we were unable to demonstrate eosinophil degranulation as measured by EPO activity in cell-free BAL fluid or in the EPO content per eosinophil. It remains possible that other eosinophil-derived mediators, such as leukotrienes, are involved in the induction of hyperreactivity *in vivo*.

The SP-induced hyperreactivity was not accompanied by changes in the number of eosinophils or other leucocytes in BAL fluid. In addition, SP did not seem to induce eosinophil degranulation, since EPO activity in BAL fluid and EPO content per eosinophil were not altered. It seems unlikely that SP directly affected the responsiveness of airway smooth muscle to histamine, since incubation of tracheal rings with SP *in vitro* did not lead to hyperreactivity. However, it is difficult to directly compare *in vitro* and *in vivo* findings.

Since IL-5- and SP-induced hyperreactivity after intra-airway administration was not additive, the mechanism of action may be similar. A possible explanation would be that SP is a common downstream mediator in the sequence of events leading to airway hyperreactivity. In agreement with this, it has been shown that depletion of sensory neuropeptides, including SP, by capsaicin treatment prevents not only ovalbumin-induced hyperreactivity [7, 8] but also hyperreactivity after platelet-activating factor [29], toluene diisocyante [30], delayed type hypersensitivity reactions [31], or respiratory viral infection [32].

Based on these data, it can be hypothesized that IL-5 is predominantly involved in the recruitment of eosinophils, whereas SP is involved in the development of hyperreactivity. At least two possible explanations can be suggested for the link between the role of eosinophils and the role of SP in the development of ovalbumin-induced hyperreactivity. Firstly, SP may be involved in the activation of eosinophils which may already be primed by cytokines, such as IL-5. Although few data are available on the activation of eosinophils by SP, KROEGEL *et al*. [33] demonstrated that SP can induce EPO release from guinea-pig eosinophils. In contrast, we were unable to demonstrate EPO release after *in vivo* treatment with IL-5 and SP.

Secondly, infiltrated and subsequently activated eosinophils may trigger sensory nerve endings inducing the release of SP and other neuropeptides. The mechanism by which eosinophils may trigger sensory nerves is at present unknown. Released cationic granule proteins seem to be likely mediators, since it has recently been shown that they can induce the release of neuropeptides in human bronchi, indicating the activation of sensory nerves [34]. In addition, intratracheal instillation of cationic proteins induced airway hyperreactivity in rats, which could be antagonized by pretreatment with a SP receptor antagonists [34]. The role of neuropeptides is further substantiated by the demonstration that release of endogenous sensory neuropeptides elicited by inhalation of capsaicin can induce airway hyperreactivity in guinea-pigs on the following day [35]. Interestingly, it has recently been shown that a neurokin-2 (NK_2) receptor antagonist prevents ovalbumin-induced airway hyperreactivity in sensitized guinea-pigs [19]. In line with these data, it has been demonstrated that airway nerves are surrounded by and infiltrated with eosinophils after ovalbumin challenge, which is consistent with an effect of eosinophils on neural function [36].

These data collectively suggest an important downstream role for neuropeptides in the development of ovalbumin-induced airway hyperreactivity. IL-5 may be a more upstream mediator, primarily involved in the recruitment and priming of eosinophils. However, the precise interactions between IL-5, eosinophils and SP in the development of hyperreactivity awaits further investigation.

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