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Modulation of airway hyperresponsiveness and eosinophilia by selective histamine and 5-HT receptor antagonists in a mouse model of allergic asthma

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1 Since both histamine and 5-hydroxytryptamine (5-HT) can be released by murine mast cells, we investigated the possible role of these autacoids on airway hyperresponsiveness (AHR), eosinophil infiltration and serum-IgE levels in a murine model of allergic asthma.

2 Ovalbumin-sensitized mice were exposed to either ovalbumin (2 mg ml^{-1}) or saline aerosols on 8 consecutive days. Starting one day before the challenge, animals were injected i.p. twice a day with a 5-HT-type 1 (5-HT_1) or type 2 (5-HT_2) receptor antagonist (methiotepine, 1.25 or 2.0 mg kg^{-1} and ketanserin, 12 mg kg^{-1}, respectively) or a histamine-type 1 (H_1) or type 2 (H_2) receptor antagonist (mepyramine, 12 or 20 mg kg^{-1} and cimetidine, 10 or 25 mg kg^{-1}, respectively). Furthermore, animals were injected with a combination of cimetidine and ketanserin or with an α-adrenoceptor antagonist (phentolamine, 5 mg kg^{-1}).

3 In vehicle-treated ovalbumin-challenged animals airway responsiveness to intravenous injections of methacholine *in vivo* was significantly (9 fold increase, $P<0.01$) increased when compared to vehicle-treated saline-challenged animals. Furthermore, ovalbumin challenge of vehicle-treated animals induced a significant increase in both eosinophil numbers in bronchoalveolar lavage (BAL) fluid (0 ± 0, vehicle/saline and $15.0\pm5.9\times10^4$ cells vehicle/ovalbumin, $P<0.05$) and ovalbumin-specific IgE levels in serum (157 ± 69 and 617 ± 171 units ml^{-1}, respectively, $P<0.05$) compared to saline-challenged mice. Virtually no eosinophils could be detected in saline-challenged animals after all different treatments.

4 Treatment with ketanserin or cimetidine resulted in a partial but significant decrease of the ovalbumin-induced AHR compared to ovalbumin-challenged controls ($P<0.05$) and reduced eosinophil infiltration after ovalbumin challenge by 60% and 58%, respectively. The combination of cimetidine and ketanserin almost completely abolished AHR whereas eosinophilia was decreased by 49%. No effects of these antagonists were observed on IL-16 levels in BAL fluid or on serum antigen-specific IgE levels. Treatment with either the H_1-receptor, the 5-HT_1-receptor or the α-adrenoceptor antagonist, did not decrease the observed ovalbumin-induced airway responsiveness or eosinophilia in vehicle-treated animals. Higher doses of either methiotepine (2.0 mg kg^{-1}) or mepyramine (20 mg kg^{-1}) did decrease ovalbumin-induced eosinophil infiltration (by 67%, $P<0.05$ and 73%, respectively), whereas no effects of these antagonists were observed on ovalbumin-specific IgE levels in serum.

5 From these data it can be concluded that both histamine and 5-HT play a role in antigen-induced AHR and eosinophilia in the mouse.

Keywords: Histamine; 5-hydroxytryptamine; hyperresponsiveness; eosinophils; asthma; IgE

Introduction

Upon antigen challenge IgE-mediated mast cell degranulation occurs, which leads to an immediate bronchoconstrictive reaction in patients with allergic asthma (Gomez *et al*., 1986). Besides an immediate bronchoconstriction, allergic asthma in man is characterized by antigen-specific IgE in serum, airway hyperresponsiveness and inflammation of lung tissue (Djukanovic *et al*., 1990; Cockroft & O'Byrne, 1993). Furthermore, Th_2 cells are thought to play an important regulatory role in this disease (Corrigan & Kay, 1992; see Van Oosterhout & Nijkamp, 1993). We have developed a mouse model to investigate several asthma related phenomena. Increased antigen-specific IgE levels in serum are present and non-specific airway hyperresponsiveness and eosinophil infiltration in bronchoalveolar lavage (BAL) fluid have been demonstrated (Hessel *et al*., 1995; 1997; De Bie *et al*., 1996). It has also been shown that mast cell degranulation upon antigen challenge occurs accompanied by an immediate bronchoconstriction and an increase in mucosal exudation (Hessel *et al*., 1995). Besides a role in early asthmatic reactions, mast cells have also been implicated in the development of airway hyperresponsiveness. Using mast cell deficient mice, Nagai *et al*. (1996) demonstrated that mast cells play an important role in the onset of airway hyperresponsivness. Furthermore, mast cell activation enhances antigen-induced airway hyperresponsiveness to methacholine in mice (Martin *et al*., 1993). Besides a role in airway hyperresponsiveness, mast cells are also believed to play a central role in allergic inflammation via release of cytokines, including interleukin-4 (IL-4) and IL-5 (Plaut *et al*., 1989). In contrast, in mast cell deficient mice, mast cells were shown not to be important for the induction of chronic airway inflammation (Brusselle *et al*., 1994; Nagai *et al*., 1996).

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Murine mast cells are known to release both histamine and 5-hydroxytryptamine (5-HT) upon stimulation *in vivo* (Ameisen *et al*., 1989; Dvorak *et al*., 1994). Both histamine and 5-HT have been shown to induce IL-16 production by $CD8^+$ T cells (Laberge *et al*., 1995; 1996). IL-16, previously known as LCF (Center *et al*., 1995), is a potent chemoattractant for human eosinophils (Rand *et al*., 1991) and $CD4^+$ T cells (Center *et al*., 1995), which are thought to play a central role in the pathogenesis of asthma (Corrigan & Kay, 1992). Interestingly, we have recently shown that, upon antigen-challenge, the presence of IL-16 in BAL fluid derived from ovalbumin-sensitized and challenged animals can be detected (Hessel *et al*., 1998), which is in agreement with the presence of IL-16 in human asthmatics (Laberge *et al*., 1997). In addition, treatment with neutralizing antibodies to IL-16 has an inhibitory effect on airway hyperresponsiveness in our animal model (Hessel *et al*., 1998). Other pro-inflammatory actions by histamine and 5-HT include an increase in vascular permeability, mucus production and various T-cell related effects (White, 1990; Ptak *et al*., 1991a; b; Young *et al*., 1993; Young & Matthews, 1995).

Since both histamine and 5-HT can be released by murine mast cells upon antigen challenge (Ameisen *et al*., 1989; Dvorak *et al*., 1994), we wanted to investigate the role of histamine and 5-HT in asthma-related features. We therefore measured the presence of IL-16 in BAL fluid, *in vivo* airway responsiveness to methacholine, inflammatory cell infiltration into the airways and serum levels of antigen-specific IgE after treatment with selective 5-HT or histamine receptor antagonists in a murine model of allergic asthma.

Methods

Sensitization and challenge

Specified pathogen free male BALB/c mice (age 6–8 weeks) were obtained from the breeding colony of the Central Animal Laboratory (GDL), Utrecht, The Netherlands. The mice were housed in macrolon cages and provided with food and water *ad libitum*. Active sensitization was performed by 7 intraperitoneal injections of 10 μg ovalbumin (grade V) in 0.5 ml pyrogen-free saline on alternate days. This sensitization procedure has been shown to induce high titres of total immunoglobulin E antibodies in the serum, of which 80% was ovalbumin-specific (Hessel *et al*., 1995). Four weeks after the last injection, the mice were exposed either to 8 ovalbumin (2 mg ml^{-1} in saline) or to 8 saline aerosols for 5 min, on consecutive days (1 aerosol per day). The aerosols were generated with a jet nebulizer (Pari IS-2, Pari-Werk GmbH, Starnberg, Germany) connected to a plexiglas exposure chamber with a volume of 5 l in which a maximum of 6 animals were placed. One day before the challenge started and during the entire challenge period, the mice were injected intraperitoneally twice a day with selective 5-HT-type 1 (5-HT_1) or type 2 (5-HT_2) receptor antagonists (methiotepine, 1.25 mg kg^{-1} or 2.0 mg kg^{-1}; ketanserin, 12 mg kg^{-1}) or selective histamine-type 1 (H_1) or type 2 (H_2) receptor antagonists (mepyramine, 12 mg kg^{-1} or 20 mg kg^{-1}; cimetidine, 10 mg kg^{-1} or 20 mg kg^{-1}). Doses used were adapted from other studies in which mice were treated with these receptor antagonists (Oishi *et al*., 1993; Garssen *et al*., 1993; Shaoheng & Walls, 1997) or according to the manufacturer's advice. Furthermore, animals were injected with a combination of cimetidine (10 mg kg^{-1}) and ketanserin (12 mg kg^{-1}) or with an α-adrenoceptor antagonist (phentolamine, 5 mg kg^{-1}) since ketanserin also has effects on these receptors (Cazzola *et al*., 1990). Control mice were injected with 0.25 ml sterile saline.

Airway responsiveness in vivo

Airway responsiveness was measured *in vivo* 24 h after the last aerosol exposure using a modified plethysmograph as described by Corry *et al*. (1996). In short: mice were anaesthetized by intraperitoneal injection of urethane (2 g kg^{-1}), and placed on a heated blanket (30°C). Then, the trachea was cannulated and a small polyethylene catheter was placed in the jugular vein for intravenous administrations. Spontaneous breathing of the animals was suppressed by intravenous injection of tubocurarine chloride (3.3 mg kg^{-1}). When the breathing stopped, the tracheal cannula was attached to a ventilator (C.F. Palmer, London, U.K.). The inflation volume of the ventilator was 0.8 ml of which the mouse inhales approximately 0.15 ml per breath with a rate of 200 breaths min^{-1}. Under these conditions, mice maintain physiological arterial blood gas parameters (data not shown). Changes in resistance were measured by use of a plethysmograph, coupled to a pressure transducer (M45, Validyne Engineering Corp., Northridge, CA, U.S.A.). By use of a pulmonary mechanics analyzer (Model 6, Buxco Corp., Sharon, CT, U.S.A.), lung resistance (R_L) was measured by quantitating $\Delta P_t \cdot \Delta V^{-1}$ (ΔP_t = change in tracheal pressure, ΔV = change in flow) at points of equal volume (70% tidal volume). Changes in tracheal pressure were measured using a pressure transducer connected to the tracheal ventilation cannula, changes in flow were measured by use of a pressure transducer connected to the plethysmograph (pressure changes were calibrated to changes in volume over the physiologic range studied). At time intervals of at least 4 min and after the response had returned to baseline level, doses of methacholin ranging from 40 μg kg^{-1} to 640 μg kg^{-1} were administered via the jugular catheter. Concentrations of methacholine were prepared in saline and kept on ice for the duration of the experiment. For each dose of methacholine the increase in airway resistance was measured at its peak and expressed in cmH_2O ml^{-1} s^{-1}. At least 6 mice were evaluated per experimental group.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed in the same animals that were used for airway hyperresponsiveness measurements. In pilot experiments it was found that combining these techniques had no effect on the total number of cells derived from the lavage nor on the appearance of the different cell types. Mice were lavaged 5 times through the tracheal cannula with 1 ml aliquots of pyrogen free saline at 37°C. The first aliquot was supplemented with the protease inhibitor aprotinine (2 μg ml^{-1}) and after centrifugation the supernatant of this first ml was stored at −80°C for cytokine measurements. The BAL cells were washed with cold phosphate-buffered saline (PBS) (400 g, 4°C, 5 min) and the pellet was resuspended in 200 μl cold PBS. The total number of BAL cells was counted by use of a Bürker-Türk chamber. For differential BAL cell counts cytospin preparations were made and stained with Diff-Quick (Merz & Dade A.G., Düdingen, Switzerland). After coding, all cytospin preparations were evaluated by one observer using oil immersion microscopy. Cells were identified and differentiated into mononuclear cells, neutrophils and eosinophils by standard morphology. At least 200 cells per cytospin preparation were counted and the absolute number of each cell type was calculated.

Determination of ovalbumin-specific immunoglobulin E serum levels

Ovalbumin-specific IgE was measured in serum derived from blood isolated from mice after airway responsiveness measurements were completed. Ninety-six well microplates (Nunc A/S., Roskilde, Denmark) were coated with 2 μg ml^{-1} chimeric fusion protein of the human high affinity immunoglobulin E receptor and human immunoglobulin G (FcεR1-IgG) diluted in PBS. After 12–24 h incubation at 4°C the plates were washed 5 times with PBS supplemented with 0.05% Tween-20 (PBT). Thereafter, the plates were blocked with ELISA buffer (2 mM EDTA, 136.9 mM NaCl, 50 mM Tris, 0.5% BSA, 0.05% Tween-20, pH 7.2) and left to incubate at room temperature for 1 h on an orbital shaker. Serum samples derived from ovalbumin-challenged animals were diluted 1 : 10, samples from saline-challenged animals 1 : 5 in ELISA buffer. Then, diluted serum samples were added to the plates and left to incubate on an orbital shaker at room temperature for 2 h. Ovalbumin-IgE reference standard dilution series were treated the same as the serum samples. The standard was obtained by intraperitoneal immunization of mice with ovalbumin, and arbitrarily assigned a value of 1,000 units ml^{-1} ovalbumin-specific immunoglobulin E (Holt *et al*., 1981). After washing, 10 μg ml^{-1} of ovalbumin in ELISA buffer was added to each well and after incubation at room temperature for 1 h washing procedures were repeated. Horse-radish peroxidase-conjugated goat anti-ovalbumin antibody was diluted in ELISA buffer and added to each well. Incubation was continued for 1 h followed by washing procedures. The last step was a 15–30 min incubation at room temperature with 10 mM OPD substrate solution after which the reaction was stopped by adding H_2SO_4 (4 M). OD was read at λ 492 nm using a Titertek Multiskan (Flow Labs., Irvine, U.K.).

IL-16 migration assay

Since IL-16 levels in BAL are too low for detection in a specific ELISA (detection limit 40 pg ml^{-1}) a modified Boyden chemotaxis chamber was used for IL-16 measurements (Cruikshank & Center, 1982). In short; human lymphocytes were isolated from heparin-treated venous blood samples of healthy normal volunteers by density centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.). The cell layer containing peripheral blood mononuclear cells was recovered and washed 3 times in Medium 199 supplemented with 25 mM HEPES buffer, 100 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin. The cells were incubated on a nylon wool column at 37°C and 5% CO_2 for 45 min. The cell population eluted from the column contained >97% T-lymphocytes as determined by fluorescent staining with anti-CD3 monoclonal antibody (Becton-Dickinson, Mountain View, CA, U.S.A.). Thirty microlitres of BAL fluid derived from ovalbumin or saline-challenged mice was placed in the lower well and the lymphocytes (1×10^7 cells in 50 μl Medium 199 enriched with 0.4% bovine serum albumin) were loaded into the upper well of the Boyden chamber. For blocking experiments, rabbit-anti-human IL-16 polyclonal antibody was added also to the lower well. In previous experiments it was shown that 5 μg ml^{-1} αIL-16 antibody neutralizes 0.1 nM of recombinant human IL-16 protein. αIL-16 mABs were only added if the migration was enhanced compared to medium induced migration. The upper and lower well were separated by a nitrocellulose filter with a pore size of 8 μm. The chamber was incubated for 3 h and afterwards the filter was fixed and stained with haematoxylin. Migration was quantified by counting the number of cells that migrated beyond a depth of 50 μm utilizing an Optomax automated image analyzer (Burlington, MA, U.S.A.). All migration data are expressed as % values of cell migration in Medium 199 enriched with 0.4% bovine serum albumin, which was normalized to 100%. All BAL samples were tested in triplicate.

Drugs and chemicals

Ovalbumin (chicken egg albumin crude grade V), o-phenylenediamine, 3-amino-1,2,4-triazole and mepyramine were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.), recombinant human FcεR1-IgG, horse-radish peroxidase-conjugated goat anti-ovalbumin antibody and ovalbumin-IgE reference standard were generously provided by Dr P.M. Jardieu, Genentech Inc. (South San Francisco, CA, U.S.A.). Urethane and methacholine (acetyl-β-methylcholine) were purchased from Janssen Chimica (Beerse, Belgium), tubocurarine chloride from Nogepha (The Netherlands), Tween-20 from Merck (Darmstadt, Germany) and phentolamine from Ciba Geigy (Basel, Switzerland). Cimetidine was purchased from SmithKline Beecham (Irvine, U.K.) and ketanserin and methiotepine from ICN (Costa Mesa, CA, U.S.A.).

Statistical analyses

Whole concentration-response curves, total BAL cell number and the numbers of the various BAL cell types were tested with an analysis of variance (ANOVA). For cell types with a very low number in control animals (i.e. neutrophils and eosinophils) a Poisson distribution was assumed. Comparisons between 2 groups were made by a Student's t test (unpaired, two-tailed). Data are expressed as arithmetic average $\pm$ s.e.-mean and a difference was considered to be significant when $P<0.05$. Statistical analyses were carried out using SPSS/PC$^+$, version 4.0.1 (SPSS Inc., Chicago, IL, U.S.A.) or GLIM, version 4.0 (NaG Inc., Oxford, U.K.).

Results

Airway resistance in vitro

Intravenous methacholine (480 μg kg^{-1}) produced a 5 fold increase in airway responsiveness after ovalbumin challenge in vehicle-treated animals ($P<0.05$, Student's t test), whereas a 9 fold increase was observed both at 560 μg kg^{-1} and at 640 μg kg^{-1} methacholine, compared to vehicle-treated saline-challenged mice ($P<0.01$) (Figure 1). Cimetidine-treated (10 mg kg^{-1}) ovalbumin-challenged animals showed a partial but significant decrease in airway hyperresponsiveness compared to vehicle-treated ovalbumin-challenged mice (Figure 1). At the highest dose of methacholine (640 μg kg^{-1}) the observed decrease was 49% ($P<0.05$). However, airway hyperresponsiveness was still present ($P<0.05$) compared to cimetidine-treated saline-challenged animals. The dose of cimetidine used in this study inhibited histamine-induced decrease in blood pressure (data not shown). Ketanserin-treated animals (12 mg kg^{-1}) also showed a partial but significant decrease in airway responsiveness compared to vehicle-treated ovalbumin-challenged mice (Figure 1). At the highest dose of methacholine (640 μg kg^{-1}) the decrease was 36% ($P<0.05$). However, this decrease was still significantly enhanced compared to ketanserin-treated saline-challenged animals ($P<0.05$) (Figure 1). Higher doses of ketanserin could

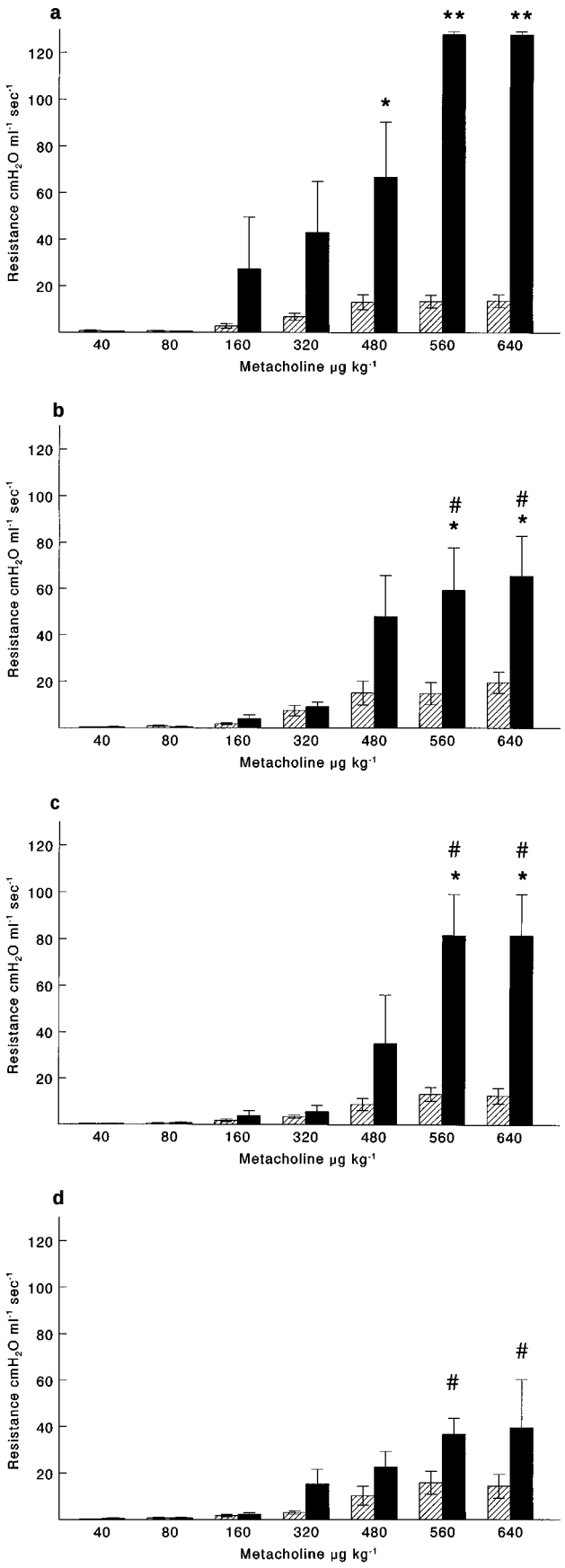

Figure 1 Airway responsiveness to intravenous administration of methacholine in ovalbumin-sensitized mice at 24 h after the last challenge with saline (hatched columns) or ovalbumin (solid columns) and treated with vehicle (a), cimetidine (10 mg kg^{-1}) (b), ketanserin (12 mg kg^{-1}) (c) or a combination of cimetidine (10 mg kg^{-1}) and ketanserin (12 mg kg^{-1}) (d). Results are expressed as arithmetic average ± s.e.mean ($n=6$ per group). Significantly different from saline-challenged animals: *$P<0.05$, **$P<0.01$, Student's t test. #Significantly different from vehicle-treated ovalbumin-challenged animals ($P<0.05$, Student's t test).

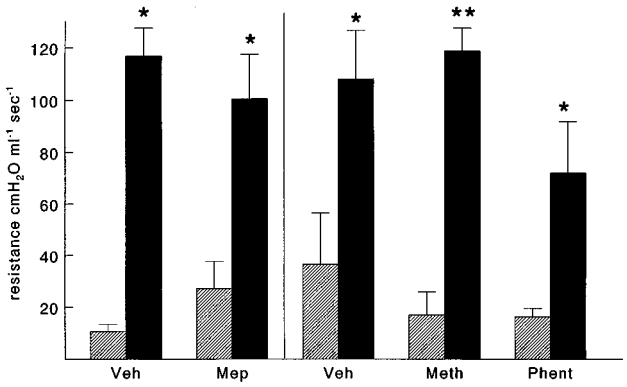

Figure 2 Increase in airway resistance at 640 $\mu g\ kg^{-1}$ of methacholine in ovalbumin-sensitized and saline (hatched columns) or ovalbumin (solid columns) challenged animals at 24 h after the last challenge. Animals were intraperitoneally injected with either vehicle (Veh), methiotepine (1.25 mg kg^{-1}) (Meth) or phentolamine (5 mg kg^{-1}) (Phent) or in a different experiment with vehicle (Veh) or mepyramine (12 mg kg^{-1}) (Mep) during the challenge period. Results are expressed as arithmetic average ± s.e.mean ($n=6$ per group). Significantly different from saline-challenged animals: *$P<0.05$, **$P<0.01$, Student's t test.

not be used to inhibit AHR further since it was observed that ketanserin has sedative actions in mice. No airway hyperresponsiveness could be observed in animals treated with a combination of ketanserin (12 mg kg^{-1}) and cimetidine (10 mg kg^{-1}) compared to their saline-challenged controls. This combination caused a decrease in airway responsiveness of 69% which was significantly different from vehicle-treated ovalbumin-challenged animals ($P<0.05$) (Figure 1).

Complete dose-response curves were also made in animals treated with either a selective H_1-receptor antagonist (mepyramine, 12 mg kg^{-1}), a 5-HT_1-receptor antagonist (methiotepine, 1.25 mg kg^{-1}) or an α-adrenoceptor antagonist phentolamine (5 mg kg^{-1}) (results not shown). At the highest dose of methacholine (640 $\mu g\ kg^{-1}$) a significant airway hyperresponsiveness to methacholine was observed in vehicle-treated mice after ovalbumin challenge ($P<0.05$) compared to saline-challenged animals (Figure 2). Treatment with either the H_1-receptor, the 5-HT_1-receptor or the α-adrenoceptor antagonist, did not decrease the observed ovalbumin-induced airway hyperresponsiveness (Figure 2).

Eosinophil infiltration

Cells in bronchoalveolar lavage fluid of animals collected at 24 h after the last challenge were differentiated by light microscopy. No differences in infiltration of mononuclear cells or neutrophils between all different antagonist treatments could be detected after either ovalbumin or saline challenge (Tables 1 and 2). Virtually no eosinophils could be detected in BAL fluid derived from saline-challenged animals treated with vehicle, ketanserin, cimetidine or the combination of antagonists (Tables 1 and 2). After ovalbumin challenge of vehicle-treated animals a significant migration of eosinophils into the BAL fluid was observed ($15.0 \pm 5.9 \times 10^4$ cells; $P<0.05$, ANOVA). Treatment with either cimetidine

(10 mg kg^{-1}) or ketanserin (12 mg kg^{-1}) diminished the infiltration of eosinophils with 60% ($6.2 \pm 3.9 \times 10^4$ cells) and 58% ($6.6 \pm 2.7 \times 10^4$ cells), respectively, compared to the number of eosinophils present in BAL fluid of vehicle-treated ovalbumin-challenged animals. The combination of cimetidine and ketanserin inhibited the eosinophil infiltration with 49% ($8.0 \pm 5.0 \times 10^4$ cells) (Table 1). The higher dose of cimetidine (25 mg kg^{-1}) did not further diminish eosinophil infiltration (data not shown).

No differences were observed in eosinophil infiltration between vehicle-treated ovalbumin-challenged animals and methiotepine-, phentolamine-, or mepyramine-treated ovalbumin-challenged animals (Table 2). However, higher doses of either methiotepine (2.0 mg kg^{-1}) or mepyramine (20 mg kg^{-1}) did decrease eosinophil infiltration with 67% ($1.7 \pm 1.3 \times 10^5$ cells; $P = 0.09$, ANOVA) and 73% ($1.4 \pm 1.0 \times 10^5$ cells; $P < 0.05$, ANOVA) when compared to eosinophil numbers in BAL fluid derived from vehicle-treated ovalbumin-challenged animals ($5.3 \pm 1.4 \times 10^5$ cells).

Table 1 Number of leukocytes in bronchoalveolar lavage fluid

Treatment	*Challenge*	*Mononuclear cells* ($\times 10^5$)	*Eosinophils* ($\times 10^4$)	*Neutrophils* ($\times 10^4$)
Vehicle	Sal	3.0±0.4	0.0±0.0	0.1±0.1
	Ova	3.3±0.7	15±5.9*	0.0±0.0
Cimetidine	Sal	2.9±0.4	0.0±0.0	0.1±0.1
	Ova	2.9±0.5	6.2±3.9*	0.0±0.0
Ketanserin	Sal	2.6±0.3	0.0±0.0	0.0±0.0
	Ova	2.6±0.4	6.6±2.7*	0.1±0.0
Cimetidine + ketanserin	Sal	2.5±0.2	0.1±0.1	0.1±0.0
	Ova	2.4±0.5	8.0±5.0*	0.1±0.1

Total number of various cell types in BAL fluid recovered at 24 h after the last ovalbumin (Ova) or saline (Sal) challenge in ovalbumin-sensitized mice treated with vehicle, cimetidine (10 mg kg^{-1}), ketanserin (12 mg kg^{-1}) or a combination of ketanserin (12 mg kg^{-1}) and cimetidine (10 mg kg^{-1}). Results are expressed as arithmetic average±s.e.mean ($n = 6$ per group). *Significantly different from saline-challenged animals ($P < 0.05$, ANOVA).

Table 2 Number of leukocytes in bronchoalveolar lavage fluid

Treatment	*Challenge*	*Mononuclear cells* ($\times 10^5$)	*Eosinophils* ($\times 10^3$)	*Neutrophils* ($\times 10^3$)
Vehicle	Sal	2.4±0.3	0.0±0.0	0.3±0.3
	Ova	3.1±0.4	6.9±1.5*	0.0±0.0
Methiotepine	Sal	2.7±0.2	0.0±0.0	0.0±0.0
	Ova	2.5±0.1	6.0±2.0*	0.2±0.2
Phentolamine	Sal	2.5±0.4	0.0±0.0	0.2±0.2
	Ova	2.4±0.2	6.7±2.4*	0.3±0.2
Vehicle	Sal	2.3±0.1	0.0±0.0	0.2±0.1
	Ova	2.1±0.3	6.6±2.7*	0.0±0.0
Mepyramine	Sal	2.0±0.4	0.0±0.0	0.0±0.0
	Ova	2.1±0.3	6.9±2.2*	0.0±0.0

Total number of various cell types in BAL fluid recovered at 24 h after the last ovalbumin (Ova) or saline (Sal) challenge in ovalbumin-sensitized mice treated with vehicle, methiotepine (1.25 mg kg^{-1}) or phentolamine (5 mg kg^{-1}). In a different experiment, animals were treated with vehicle or mepyramine (12 mg kg^{-1}). Results are expressed as arithmetic average±s.e.mean ($n = 6$ per group). *Significantly different from saline-challenged animals ($P < 0.05$, ANOVA).

Ovalbumin-specific IgE production

Serum levels of ovalbumin-specific immunoglobulin-E were determined in animals treated with vehicle, cimetidine (10 mg kg^{-1}), ketanserin (12 mg kg^{-1}) or with a combination of ketanserin (12 mg kg^{-1}) and cimetidine (10 mg kg^{-1}). A significant increase in the level of ovalbumin-specific IgE was found after ovalbumin challenge of vehicle-treated animals ($P < 0.05$, Student's t test) (Table 3). No significant differences were observed between the ovalbumin-induced increase in serum ovalbumin-IgE levels in the groups of mice treated with different antagonists. Furthermore, no significant differences were observed between the ovalbumin-induced increase in ovalbumin-specific IgE levels in serum obtained from animals treated with the higher doses of either mepyramine or methiotepine (data not shown).

IL-16 levels in BAL

Migration of human lymphocytes induced by BAL fluid collected from vehicle-treated saline-challenged animals was significantly less (87±3%) than migration induced by medium (100%; $P < 0.05$, modified Student's t test), indicating that inhibitory factors are present in these BAL fluid samples. BAL fluid samples from ovalbumin-challenged vehicle-treated animals induced a significant increase (219±21%; $P < 0.05$; Student's t test) in lymphocyte migration compared to both medium controls and BAL fluid from saline-challenged vehicle-treated animals. Of this migration 80±21% appeared to be due to IL-16, as determined by neutralizing polyclonal antibodies. BAL fluid derived from ovalbumin-challenged animals treated with either cimetidine, ketanserin or the combination of these two antagonists did not induce a significantly different lymphocyte migration when compared to BAL fluid derived from vehicle-treated ovalbumin-challenged animals (Table 4). Furthermore, no significant differences were observed in the IL-16 dependent part of this migration. Finally, no differences were observed between the lymphocyte migration, and the IL-16 attributable part of this migration, induced by BAL fluid samples obtained from saline-challenged animals of all different treatments (Table 4).

Discussion

Both the selective 5-HT_2 receptor antagonist ketanserin and the H_2 receptor antagonist cimetidine were capable of partially

Table 3 Serum ovalbumin-specific IgE levels

Treatment	*Saline*	*Ovalbumin*
Vehicle	157±69	619±171*
Cimetidine	235±84	960±182*
Ketanserin	377±118	801±223
Combination	402±122	1141±220*

Ovalbumin-specific immunoglobulin E (IgE) levels (arbitrary units ml^{-1}) measured by ELISA in serum after saline or ovalbumin challenge in ovalbumin-sensitized mice after treatment with vehicle, cimetidine (10 mg kg^{-1}), ketanserin (12 mg kg^{-1}) or a combination of cimetidine (10 mg kg^{-1}) and ketanserin (12 mg kg^{-1}). Results are expressed as arithmetic average±s.e.mean (n is at least 10 per group). *Significantly different from immunoglobulin E level in serum from saline-challenged animals ($P < 0.05$, Student's t test).

Table 4 Migration of lymphocytes induced by BAL fluid samples

Treatment	*Total migration*	*IL16-dependent part of migration*
Vehicle/Sal	87±3%	0±0%
Vehicle/Ova	219±21%	80±21%
Cimetidine/Sal	153±41%	36±30%
Cimetidine/Ova	176±42%	79±35%
Ketanserin/Sal	108±16%	13±12%
Ketanserin/Ova	198±51%	53±27%
Combination/Sal	112±15%	7±6%
Combination/Ova	214±46%	99±27%

Migration of lymphocytes induced by BAL fluid samples derived from saline (Sal) or ovalbumin (Ova)-challenged animals treated with either vehicle, cimetidine (10 mg kg^{-1}), ketanserin (12 mg kg^{-1}) or a combination of cimetidine (10 mg kg^{-1}) and ketanserin (12 mg kg^{-1}). Furthermore, the IL-16-dependent part of the induced migration is depicted. Results are expressed as arithmetic average±s.e.-mean (*n* is at least 5 per group). *Significantly different from migration induced by BAL fluid derived from saline-challenged vehicle-treated animals ($P<0.05$, Student's *t* test).

decreasing airway hyperresponsivenes and eosinophil infiltration, whereas a combination of ketanserin and cimetidine completely inhibited airway hyperresponsiveness without further decreasing eosinophil numbers in BAL fluid. No effects of either antagonist alone or the combination of antagonists on ovalbumin-specific IgE serum levels or on IL-16 levels in BAL fluid could be detected.

Previously we have developed a murine model of allergic asthma that shows several characteristics which are also observed in human allergic asthma. Non-specific airway hyperresponsiveness, eosinophil infiltration and an increase in antigen-specific serum IgE levels are commonly observed in human allergic asthma and can also be detected in this mouse model (Hessel *et al*., 1995; 1997; De Bie *et al*., 1996). Furthermore, upon antigen challenge, an early bronchoconstrictive reaction together with an increase in mucosal exudation can be measured, which is believed to be caused by mast cell degranulation after IgE cross-linking (Hessel *et al*., 1995). In man, mast cell degranulation has also been observed after antigen challenge (Gomez *et al*., 1986). This mast cell degranulation is believed to play a pivotal role in the early asthmatic reaction and is implicated in the onset of the inflammation via, for example, tumour necrosis factor-α (TNF-α), IL-4 and IL-5 release (Plaut *et al*., 1989; Bissonette, 1996). Murine mast cells have been shown to be capable of releasing both histamine and 5-HT (Ameisen *et al*., 1989; Dvorak *et al*., 1994), but little is known about the role of these autacoids in phenomena such as inflammation, IgE production and airway hyperresponsiveness. Therefore, we treated mice with several different selective histamine or 5-HT receptor antagonists to determine the role of histamine or 5-HT on these parameters.

Upon stimulation of H_2 and 5-HT_2 receptors, IL-16 is released by $CD8^+$ T cells (Laberge *et al*., 1995; 1996). IL-16 has been shown to be a very potent chemoattractant for human eosinophils (Rand *et al*., 1991) and $CD4^+$ T cells (Center *et al*., 1995), which are believed to contribute to airway hyperresponsiveness and inflammation (Corrigan & Kay, 1992; see Van Oosterhout & Nijkamp, 1993). In agreement herewith, the development of ovalbumin-induced airway hyperresponsiveness was partially impaired by treatment with monoclonal antibodies to IL-16 (Hessel *et al*., 1998). However, eosinophilia was not affected. Therefore, a possible explanation for the observed effects of cimetidine and ketanserin on airway hyperresponsiveness could be that the release of IL-16 is blocked by the antagonists used in this study. However, in the present study IL-16 levels in BAL fluid after ovalbumin challenge were not decreased by treatment with either antagonist alone or the combination. Thus, it is not very likely that the observed effects on airway hyperresponsiveness were caused by a decrease in IL-16 release. Besides $CD8^+$ T cells, epithelial cells are a well known source of IL-16 in asthmatics (Laberge *et al*., 1997). Furthermore, IL-16 can also be produced by mitogen and antigen-stimulated mononuclear cells (Center *et al*., 1996), and it is therefore possible that IL-16 levels in BAL were not decreased by H_2 and 5-HT_2 antagonists.

As was previously stated, mast cells can secrete both histamine and 5-HT (Ameisen *et al*., 1989; Dvorak *et al*., 1994) and mast cells degranulate upon antigen challenge in our model (Hessel *et al*., 1995). Furthermore, it is well known that mast cells are important in the onset of airway hyperresponsiveness (Martin *et al*., 1993; Nagai *et al*., 1994). Together with the observed effects of both cimetidine and ketanserin on airway hyperresponsiveness, it is very tempting to speculate that mast cells are involved in development of airway hyperresponsiveness via release of both histamine and 5-HT upon antigen challenge. Our observation that treatment with ketanserin can decrease airway hyperresponsiveness to methacholine is in agreement with observations made by Cazzola *et al*. (1990), who demonstrated that 5-HT_2 receptor blocking can cause a small but significant modification of airway hyperresponsiveness in human asthmatic patients. Besides being a 5-HT_2 receptor antagonist, ketanserin also has antagonistic activity on α-adrenoceptors (Rang *et al*., 1995). However, the observed inhibition of airway hyperresponsiveness in our experiments after treatment with ketanserin was 5-HT_2 receptor-specific, since no effects were observed when either a 5-HT_1 receptor antagonist or an α-adrenoceptor antagonist was used. Treatment with cimetidine resulted in a partial inhibition of both airway hyperresponsiveness and eosinophil infiltration. However, cimetidine is also capable of binding to H_1 receptors, although the affinity for H_1 receptors is less than the affinity for H_2 receptors (Rang *et al*., 1995). Furthermore, H_2 receptor antagonists prevent release of histamine and therefore it is difficult to analyse *in vivo* results of treatment with such antagonists (Rocklin & Beer, 1983). The observation that H_1 as well as 5-HT_1 receptor antagonists could reduce eosinophil infiltration at higher doses could be due to nonselective effects associated with these high doses, e.g. on other histamine or 5-HT receptors. However, these data do not exclude the involvement of H_1 and 5-HT_1 receptors in eosinophil infiltration upon antigen challenge.

An explanation for the observed effects on eosinophil infiltration and airway hyperresponsiveness could be that both histamine and 5-HT have several pro-inflammatory effects. Therefore, inhibition of the action of these autacoids could lead to a decrease in inflammation and airway hyperresponsiveness. Histamine, for example, has been shown to augment activation of cytotoxic T lymphocytes *in vivo* and production of IL-2 *in vitro* (Dröge *et al*., 1986). Furthermore, histamine has been shown to inhibit interferon γ (IFNγ) production in a H_2 receptor restricted manner (Dohlsten *et al*., 1987). IFNγ is well known for its capabilities to down-regulate Th_2-mediated responses such as eosinophil infiltration and airway hyperresponsiveness (see Van Oosterhout & Nijkamp, 1993). In contrast, histamine also has immunosuppressive capacities such as inhibition of antigen- and mitogen-induced T cell responses and production of cytokines including IL-2, IL-1

and TNFα (Bissonette, 1996). Besides lymphocyte-mediated effects histamine appears to be chemotactic for eosinophils when studied *in vivo* and *in vitro*, which is thought to be mediated via both H_1 and H_2 receptors (Rocklin & Beer, 1983). This would imply that the effects observed by either the H_1 or H_2 receptor antagonist are due to nonselective inhibition of histamine receptors, which is in agreement with, for example, the observation that mepyramine only has an effect on eosinophil infiltration when administered in high doses.

5-HT is involved in T cell activation (Young *et al*., 1993), local recruitment and activation of $CD4^+$ T helper cells (Ptak *et al*., 1991a, b) and B cell proliferation (Young & Matthews, 1995). Therefore, it could be speculated that cimetidine and ketanserin exert their activity via inhibition of lymphocyte functions. Little is known about the role of either histamine or 5-HT in antibody production by B cells. However, since antigen-specific IgE levels in serum were not affected by the different treatments it is not very likely that B cell functioning is impaired by one of the antagonists alone or by the combination of cimetidine and ketanserin.

Another characteristic that could have contributed to the effects caused by cimetidine and ketanserin, as well as the higher doses of mepyramine and methiotepine, is that both histamine and 5-HT are able to increase vascular permeability (White, 1990; Fujii *et al*., 1994). 5-HT has been clearly demonstrated to increase vascular permeability via 5-HT_2 receptor stimulation (Fujii *et al*., 1994), whereas histamine has been found mainly to induce vascular leakage upon H_1 receptor stimulation. This seems to be in contrast with our observations that an H_2 receptor antagonist caused a decrease in airway hyperresponsiveness, whereas an H_1 receptor antagonist (12 mg kg^{-1}) did not cause any effect. However, H_1 receptor antagonists alone are less able to prevent the increase in vasopermeability than combinations of H_1 and H_2 receptor antagonists in preventing the increase in vasopermeability (Rocklin & Beer, 1983). Combined with the observations that cimetidine can bind to both H_2 and H_1 receptors (Rang *et al*., 1995) and that H_2 receptor antagonists prevent histamine release (Rocklin & Beer, 1983), it could therefore be speculated that cimitidine as well as ketanserin prevented the antigen-induced increase in vasopermeability after ovalbumin challenge (Hessel *et al*., 1995). An increase in vascular permeability could not only lead to oedema of the airway wall which contributes to increased airway hyperresponsiveness (Rang *et al*., 1995), but also to leakage of plasma proteins into the airways, which leads to an increase of the viscosity of mucus (Djukanovic *et al*., 1990). This increase in viscosity retards the mucociliary clearance of mucus which constitutes an integral part of the inflammatory response (Djukanovic *et al*., 1990). Moreover, histamine is believed to play a direct stimulating role on the mucus production in the lower airways via H_2 receptor stimulation (White, 1990). It has previously been established in the used model that an ovalbumin-induced increase in mucus exudation as well as goblet cell hyperplasia occurs (Blyth *et al*., 1996). The observed mucus production could very well predispose ovalbumin-challenged animals to air trapping, especially after administration of a well known secretagogue for mucus such as methacholine. Thus, it could be possible that the effects of either the histamine or 5-HT receptor antagonist on airway hyperresponsiveness are caused by a decrease in antigen-induced mucus production or exudation.

The combination of both cimetidine and ketanserin did completely abolish airway hyperresponsiveness without further decreasing eosinophil infiltration. The observation that eosinophilia and airway hyperresponsiveness are not always closely related is in agreement with observations made both in man and animal models (Djukanovic *et al*., 1992; Hessel *et al*., 1997). The observed effects of the combination of ketanserin and cimetidine could be due to a complete inhibition of the antigen-induced increase in vascular permeability, mucus production and possibly T lymphocyte functioning by both histamine and 5-HT. However, since eosinophil infiltration was not further decreased by the combination of antagonists it is not very likely that the main effect of these antagonists is impairment of immune cell functions. If these antagonists would have impaired T lymphocyte functions such as cytokine production, eosinophil infiltration would have been completely blocked since it is generally accepted that Th_2-type cytokines are crucial for the development of eosinophilia (see Van Oosterhout & Nijkamp, 1993).

In conclusion, our data suggest that 5-HT and histamine are mediators of antigen-induced airway hyperresponsiveness and, to a lesser degree, of inflammation in the mouse. Furthermore, it can be speculated that these actions probably take place via 5-HT_2 and H_2 receptors. Since a combination of both antagonists completely inhibited airway hyperresponsiveness it can also be concluded that these antagonists act in an additive way. However, the exact mechanism by which these antagonists exert their action on asthma related features and via which receptors remains to be elucidated.

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References

AMEISEN, J.-C., MEADE, R. & ASKENASE, P.W. (1989). A new interpretation of the involvement of serotonin in delayed-type hypersensitivity. *J. Immunol.,* **142,** 3171–3179.

BISSONETTE, E.Y. (1996). Histamine inhibits tumor necrosis factor α release by mast cells through H_2 and H_3 receptors. *Am. J. Respir. Cell Mol. Biol.,* **14,** 620–626.

BLYTH, D.I., PEDRICK, M.S., SAVAGE, T.J., HESSEL, E.M. & FATTAH, D. (1996). Lung inflammation and epithelial changes in a murine model of allergic asthma. *Am. J. Respir. Cell Mol. Biol.,* **14,** 425–438.

BRUSSELLE, G.G., KIPS, J.C., TAVERNIER, J.H., VAN DER HEYDEN, J.G., CUVELIER, C.A., PAUWELS, R.A. & BLUETHMANN, H. (1994). Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin. Exp. Allergy,* **24,** 73–80.

CAZZOLA, M., ASSOGNA, G., LUCCHETTI, G., GICCHITTO, G. & D'AMATO, G. (1990). Effect of ketanserin, a new blocking agent of the 5-HT_2 receptor, on airway responsiveness in asthma. *J. Allergy Clin. Immunol.,* **45,** 151–153.

CENTER, D.M., BERMAN, J.S., KORNFELD, H., THEODORE, A.C. & CRUIKSHANK, W.W. (1995). The lymphocyte chemoattractant factor. *J. Lab. Clin. Med.,* **125,** 167–172.

CENTER, D.M., KORNFELD, H. & CRUIKSHANK, W.W. (1996). Interleukin 16 and its function as a CD4 ligand. *Immunol. Today,* **17,** 477–480.

COCKROFT, D.W. & O'BYRNE, P.M. (1993). Mechanisms of airway hyperresponsiveness. In *Bronchial Asthma: Mechanisms and Therapeutics (3rd edition)*. ed. Weiss, E.B. & Stein, M. pp. 32–42. Boston: Little Brown.

CORRIGAN, C.J. & KAY, A.B. (1992). T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today,* **13,** 501 – 507.

CORRY, D.B., FOLKESSON, H.G., WARNOCK, M.L., ERLE, D.J., MATTHAY, M.A., WIENER-KRONISH, J.P. & LOCKSLEY, R.M. (1996). Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J. Exp. Med.,* **183,** 109 – 117.

CRUIKSHANK, W.W. & CENTER, D.M. (1982). Modulation of lymphocyte migration by human lymphokines. II. Purification of a lymphotactic factor (LCF). *J. Immunol.,* **128,** 2569 – 2574.

DE BIE, J.J., HESSEL, E.M., VAN ARK, I., VAN ESCH, B., HOFMAN, G., NIJKAMP, F.P. & VAN OOSTERHOUT, A.J.M. (1996). Effect of dexamethasone and endogenous corticosterone on airway hyperresponsiveness and eosinophilia in the mouse. *Br. J. Pharmacol.,* **119,** 1484 – 1490.

DJUKANOVIC, R., LAI, C.K.W., WILSON, K.M., BRITTEN, K.M., WILSON, S.J., ROCHE, W.R., HOWARTH, P.H. & HOLGATE, S.T. (1992). Bronchial mucosal manifestations of atopy: a comparison between atopic asthmatics, atopic nonasthmatics and healthy controls. *Eur. Respir. J.,* **5,** 538 – 544.

DJUKANOVIC, R., ROCHE, W.R., WILSON, J.W., BEASLY, C.R.W., TWENTYMAN, O.P., HOWARTH, P.H. & HOLGATE, S.T. (1990). Mucosal inflammation in asthma. *Am. Rev. Respir. Dis.,* **142,** 434 – 457.

DOHLSTEN, M., SJÖGREN, H.O. & CARLSSON, R. (1987). Histamine acts directly on human T cells to inhibit interleukin-2 and interferon-γ production. *Cell. Immunol.,* **109,** 65 – 74.

DRÖGE, W., SCHMIDT, H., NICK, S. & SONSKY, B. (1986). Histamine augments interleukin-2 production and the activation of cytotoxic T lymphocytes. *Immunopharmacology,* **11,** 1 – 6.

DVORAK, A.M., TEPPER, R.I., WELLER, P.F., MORGAN, E.S., ESTRELLA, P., MONAHAN-EARLY, R.A. & GALLI, S.J. (1994). Piecemeal degranulation of mast cells in the inflammatory eyelid lesions of interleukin-4 transgenic mice: Evidence of mast cell histamine-release in vivo by diamine oxidase-gold enzyme-affinity ultrastructural cytochemistry. *Blood,* **83,** 3600 – 3612.

FUJII, E., UCHIDA, Y., TSUKAHARA, F. & MURAKI, T. (1994). 5-Hydroxytryptamine-induced increase in vascular permeability in mouse skin. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **350,** 361 – 364.

GARSSEN, J., VAN LOVEREN, H., VAN DER VLIET, H., BOT H. & NIJKAMP, F.P. (1993). T cell-mediated induction of airway hyperresponsiveness and altered lung functions in mice are independent of increased vascular permeability and mononuclear cell infiltration. *Am. Rev. Respir. Dis.,* **147,** 307 – 313.

GOMEZ, E., CORRADO, O.J., BALDWIN, D.L., SWANSTON, A.R. & DAVIES, R.J. (1986). Direct in vivo evidence for mast cell degranulation during allergen-induced reactions in man. *J. Allergy Clin. Immunol.,* **78,** 637 – 645.

HESSEL, E.M., CRUIKSHANK, W.W., VAN ARK, I., DE BIE, J.J., VAN ESCH, B., HOFMAN, G., NIJKAMP, F.P., CENTER, D.M. & VAN OOSTERHOUT, A.J.M. (1998). Involvement of IL-16 in the induction of airway hyperresponsiveness and up-regulation of IgE in a murine model of allergic asthma. *J. Immunol.,* **160,** 2998 – 3005.

HESSEL, E.M., VAN OOSTERHOUT, A.J.M., HOFSTRA, C.L., DE BIE, J.J., GARSSEN, J., VAN LOVEREN, H., VERHEYEN, A.K.C.P. & NIJKAMP, F.P. (1995). Bronchoconstriction and airway hyperresponsiveness after ovalbumin inhalation in sensitized mice. *Eur. J. Pharmacol.,* **293,** 401 – 412.

HESSEL, E.M., VAN OOSTERHOUT, A.J.M., VAN ARK, I., VAN ESCH, B., HOFMAN, G., VAN LOVEREN, H., SAVELKOUL, H.F.J. & NIJKAMP, F.P. (1997). Development of airway hyperresponsiveness is dependent on IFNγ and independent of eosinophil infiltration. *Am. J. Respir. Cell Mol. Biol.,* **16,** 325 – 334.

HOLT, P.G., ROSE, A.H., BATTY, J.E. & TURNER, K.J. (1981). Induction of adjuvant-dependent IgE responses in inbred mice: primary, secondary, and persistent IgE responses to ovalbumin and ovomucoid. *Int. Arch. Allergy Appl. Immunol.,* **65,** 42 – 50.

LABERGE, S., CRUIKSHANK, W.W., BEER, D.J. & CENTER, D.M. (1996). Secretion of IL-16 (Lymphocyte Chemoattractant Factor) from serotonin-stimulated CD8+ T cells in vitro. *J. Immunol.,* **156,** 310 – 315.

LABERGE, S., CRUIKSHANK, W.W., KORNFELD, H. & CENTER, D.M. (1995). Histamine-induced secretion of lymphocyte chemoattractant factor from CD8+ T cells is independent of transcription and translation. *J. Immunol.,* **155,** 2902 – 2910.

LABERGE, S., ERNST, P., GHAFFAR, O., CRUIKSHANK, W.W., KORNFELD, H., CENTER, D.M. & HAMID, Q. (1997). Increased expression of interleukin-16 in bronchial mucosa of subjects with asthma. *Am. J. Respir. Cell Mol. Biol.,* **17,** 193 – 202.

MARTIN, T.R., TAKEISHI, T. & KATZ, H.R. (1993). Mast cell activation enhances airway hyperresponsiveness to methacholine in the mouse. *J. Clin. Invest.,* **1993,** 1176 – 1182.

NAGAI, H., YAMAGUCHI, S., MEADA, Y. & TANAKA, H. (1996). Role of mast cells, eosinophils and IL-5 in the development of airway hyperresponsiveness in sensitized mice. *Clin. Exp. Allergy,* **26,** 642 – 647.

OISHI, R., ADACHI, N. & SAEKI, K. (1993). N alpha-methylhistamine inhibits intestinal transit in mice by central histamine H1 receptor activation. *Eur. J. Pharmacol.,* **237,** 155 – 159.

PLAUT, M., PIECE, J.H. & WATSON, C.J. (1989). Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium-ionophores. *Nature,* **339,** 64 – 67.

PTAK, W., GEBA, G.P. & ASKENASE, P.W. (1991a). Initiation of delayed-type hypersensitivity by low doses of monoclonal IgE antibody. *J. Immunol.,* **146,** 3929 – 3936.

PTAK, W., HERZOG, W.-R. & ASKENASE, P.W. (1991b). Delayed-type hypersensitivity initiation by early-acting cells that are antigen mismatched or MHC incompatible with late-acting, delayed-type hypersensitivity effector T cells. *J. Immunol.,* **146,** 469 – 475.

RAND, T.H., CRUIKSHANK, W.W., CENTER, D.M. & WELLER, P.F. (1991). CD4-mediated stimulation of human eosinophils: lymphocyte chemoattractant factor and other CD4-binding ligand elicit eosinophil migration. *J. Exp. Med.,* **173,** 1521 – 1528.

RANG, H.P., DALE, M.M. & RITTER, J.M. (1995). In *Pharmacology* (3rd edition). ed. Rang, H.P., Dale, M.M. & Ritter, J.M. New York: Churchill Livingstone.

ROCKLIN, R.E. & BEER, D.J. (1983). Histamine and immune modulation. *Adv. Intern. Med.,* **28,** 225 – 251.

SHAOENG, H. & WALLS, A.F. (1997). Human mast cell tryptase: a stimulus of microvascular leakage and mast cell activation. *Eur. J. Pharmacol.,* **328,** 89 – 97.

VAN OOSTERHOUT, A.J.M. & NIJKAMP, F.P. (1993). Role of cytokines in bronchial hyperresponsiveness. *Pulm. Pharmacol.,* **6,** 225 – 236.

WHITE, M.V. (1990). The role of histamine in allergic diseases. *J. Allergy Clin. Immunol.,* **86,** 599 – 605.

YOUNG, M.R.I., KUT, J.L., COOGAN, M.P., WRIGHT, M.A., YOUNG, M.E. & MATTHEWS, J. (1993). Stimulation of splenic T-lymphocyte function by endogenous serotonin and by low-dose exogenous serotonin. *Immunology,* **80,** 395 – 400.

YOUNG, M.R.I. & MATTHEWS, J.P. (1995). Serotonin regulation of T-cell subpopulations and of macrophage accessory function. *Immunology,* **84,** 148 – 152.

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