

ORIGINAL PAPER

Interference of methysergide, a specific 5-hydroxytryptamine receptor antagonist, with airway chronic allergic inflammation and remodelling in a murine model of asthma

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Clinical and Experimental Allergy

Summary

Background Airway remodelling encompasses the structural changes observed in asthmatic airways. Mast cells, through the release of histamine and 5-hydroxytryptamine (serotonin), are implicated in early asthmatic reactions, bronchoconstriction and mucosal oedema, and in the development of bronchial hyperresponsiveness. However, the association between serotonin and remodelling processes in murine model of airways inflammation remains to be elucidated.

Objective As serotonin is released by murine mast cells upon antigen challenge, we tested the hypothesis of its involvement in the development of inflammatory and remodelling processes in a murine model of chronic airway inflammation following prolonged allergen challenge.

Methods BALB/c mice were exposed to aerosolized ovalbumin for 20 min 2 days a week, for 4 consecutive weeks. Two hours before each challenge, they were treated with methysergide (intranasally, 40 µg/kg). Forty-eight hours after the last aerosol challenge, bronchoalveolar lavage (BAL) and lung tissue were collected for analysis.

Results Methysergide inhibited the allergen-induced increase in airway eosinophilia, reduced T helper type 2 (Th2) cytokines in lung, spleen or thoracic lymph nodes, and specific IgE levels. The extravasation of plasma and fibronectin production in the lung, and collagen deposition in the lung were also inhibited after methysergide treatment. Although methysergide treatment induced an increase in IFN-γ levels, experiments with neutralizing antibody suggest that this is not responsible for inhibition. In addition, instillation of serotonin to immunized mice induced eosinophil recruitment to BAL, Th2 cytokine production and fibronectin release in lung as well as collagen deposition.

Conclusion Serotonin may contribute to the development and maintenance of remodelling through the release of cytokines and of fibrogenic mediators. Serotonin should therefore be considered as relevant for the development and maintenance of airway remodelling.

Keywords asthma, 5-hydroxytryptamine, lung remodelling, methysergide, prolonged allergen exposure

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Introduction

Airway remodelling encompasses the structural changes observed in asthmatic airways. These changes include goblet cell hyperplasia, increased mitogenic activity in the epithelium, and changes in extracellular matrix (ECM) components. The latter involve deposition of interstitial

collagens and fibronectin in the lamina reticularis, matrix deposition in the submucosa, smooth muscle hyperplasia, and microvascular proliferation [1–5]. The clinical consequences of airway remodelling in asthma remain uncertain, but contributions to bronchial hyperresponsiveness (BHR) and fixed airflow obstruction have been suggested [6].

Eosinophils are associated with airway remodelling in a number of settings [7, 8]. In addition, eosinophil infiltration and the expression of TGF- β by these cells can be reduced by anti-IL-5 treatment in moderate asthmatics [9] or by budesonide administration in animal models [10]. These changes were accompanied by a significant reduction in sub-basement membrane deposition of ECM proteins pro-collagen III. However, in a sheep model of chronic airway remodelling in response to house dust mite, Snibson *et al.* [11] found that 8–9 days after the last allergen challenge, eosinophils were absent and mast cell density was increased, suggesting that persistent exposure to allergen maintains chronic inflammation and might drive remodelling.

Mast cells, through the release of histamine and 5-hydroxytryptamine (5-HT, serotonin) [12], are implicated in early asthmatic reactions, bronchoconstriction, and mucosal oedema, and in the development of BHR [13–15]. The wide variety of 5-HT-mediated cell functions is regulated by expression of different classes of G protein-coupled as well as ionotropic serotoninergic receptors (5-HTR) [16, 17]. Isolated smooth muscle and endothelial cells from pulmonary arteries or primary human alveolar epithelial cells type II and the human epithelial lung cell lines A549 and BEAS-2B express mRNAs for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT₇ receptors [18–20]. A broad variety of cell responses such as migration, phagocytosis, superoxide anion generation, and cytokine production by serotonin have been reported [16, 20–24], but the association between serotonin and remodelling processes in asthmatic airways remains to be elucidated.

As serotonin is released by murine mast cells upon antigen challenge, we tested the hypothesis of its involvement in the development of inflammatory and remodelling processes. Most of the currently developed animal models of allergic airway inflammation have been restricted to acute inflammatory changes following relatively short periods of allergen exposure. In this study, we investigated whether the exposition of BALB/c mice to prolonged allergen challenge triggers airway remodelling and the role of serotonin in this processes.

Methods

Animals

Seven- to 8-week-old male BALB/c mice and 3-month-old male Wistar rats were obtained from a colony at the Butantan Institute, Sao Paulo, Brazil. The animals were maintained at the animal house facilities under specific pathogen-free conditions. All the procedures involving animals were in accordance with the guidelines provided by the Brazilian College of Animal Experimentation.

Mice sensitization

On the first day of the experiments (day 0) and on day 7, mice ($n = 6$) were immunized by an intraperitoneal (i.p.) injection of 10 μ g of ovalbumin (OVA, Grade V, Sigma-Aldrich Co., St Louis, MO, USA), adsorbed to 1.6 mg of aluminium hydroxide [Al(OH)₃]. Starting on day 14, they were exposed to aerosolized OVA (Grade V, 1%) for 20 min twice a week for 4 consecutive weeks in an adapted chamber (vehicle-treated OVA/OVA). Immunized mice challenged with phosphate-buffered saline (PBS) were considered to be the control group (OVA/PBS). The aerosol was produced by an ultrasonic nebulizer (Icel US-800, São Paulo, Brazil) as described elsewhere [25]. According to the specifications of the manufacturer, the output of the nebulizer was 2 mL/min and the mean particle size was 3.2 μ m.

To confirm our hypothesis of the involvement of serotonin in remodelling, we reproduced a chronic model of airway inflammation. In some experiments, sensitized or non-sensitized mice were challenged intra nasally (i.n.) with vehicle (40 μ L, 0.9% w/v NaCl-saline) or serotonin in the vehicle (1 μ g/mL, 40 μ L, OVA/serotonin). This dose of serotonin was derived from Little *et al.* [26]. Animals were exposed twice a week for 4 consecutive weeks. Twenty-four hours after OVA or serotonin challenge, mice were killed for bronchoalveolar lavage (BAL) and lung collection.

Treatment of mice

A group of mice were treated by an i.n. injection of vehicle (40 μ L, 10% tartaric acid diluted in saline) or 40 μ L of methysergide in the vehicle (Sandoz, Bâle, Switzerland, 40 μ g/kg dissolved in 10% tartaric acid diluted in saline) 2 h before each challenge [27, 28]. IFN- γ blocking antibody (XMG1.2; 100 μ g/animal) was injected i.p. Control rat IgG monoclonal antibody (mAb) (GL113; 100 μ g/animal) injected 1 h before each challenge was used for comparison. All antibodies were purchased from BD Pharmingen (San Jose, CA, USA).

Bronchoalveolar lavage analysis

Forty-eight hours after the last challenge with aerosolized OVA, mice were killed with an overdose of chloral hydrate and the tracheas were cannulated. The airway lumina were washed with 2×0.5 mL of Hank's balanced salt solution (HBSS, Gibco BRL, Grand Island, NY, USA) + 10 mM ethylenediaminetetraacetic acid (EDTA). The resulting BAL was immediately centrifuged at 300 g 4 °C, for 10 min. BAL cells were washed twice with HBSS containing 0.1% newborn calf serum (Sigma Chemicals Co., St Louis, MO, USA). Cell counts were performed using a haemocytometer and cytocentrifuge slides were

prepared, air-dried, fixed in methanol, and stained (Wright-Giemsa, Scientific Products, Chicago, IL, USA). For differential cell counts, 300 leukocytes were enumerated and identified as mononuclear cells, neutrophils, or eosinophils, on the basis of staining and morphologic characteristics. After BAL collection, the lungs were then removed, weighed, and homogenized in HBSS and centrifuged (1000 g, 10 min). After lyses of red blood cells, the supernatant were stored for further analysis.

Cell culture

Forty-eight hours after the last challenge with aerosolized OVA, mice were killed, and the spleens and thoracic lymph nodes (TLN) were obtained by careful dissection after the lungs had been removed from the thoracic cavity and passed through Nytex (Cadisch Precision Meshes Ltd., London, UK) to prepare a single-cell suspension. Cells were cultured at 2×10^6 cells/mL in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin, 25 mM HEPES buffer, and 10% heat-inactivated fetal calf serum (all from Life Technologies, Paisley, UK). Cells were cultured with 500 µg/mL OVA for 72 h, and the supernatants from parallel triplicate cultures were stored at -70°C until analysis of cytokine concentrations by ELISA.

Passive cutaneous anaphylaxis

The IgE antibody response was also measured by passive cutaneous anaphylaxis (PCA) as described by Mota and Wong [29]. PCA reactions were triggered in rats, using a sensitization period of 18–24 h and OVA in Evan's blue solution for challenge. The PCA titre was expressed as the reciprocal of the highest dilution that induced a lesion of more than 5 mm in diameter in triplicate tests. The detection threshold of the technique was established at 1 : 5 dilutions.

Explants

The lungs from immunized mice treated or not with methysergide were collected 48 h after the last challenge with aerosolized OVA and prepared for organ culture. Lungs were obtained under sterile conditions and cultured as described by Proust et al. [30]. Lung lobes were separated in PBS (127 mM NaCl, 10 mM Na_2HPO_4 , pH 7.4) and cultured pooled in a 24-well plate (Falcon-Becton Dickinson, Lincoln Park, NJ, USA) at 37°C in 5% CO_2 /air. The medium, Dulbecco's modified eagle medium (GibcoBRL-Life Technologies, Basel, Switzerland), was enriched with 10% serotonin-depleted serum (GibcoBRL-Life Technologies, Basel, Switzerland), 5 µg/mL bovine insulin (Sigma Chemicals Co., St Louis, MO, USA), 2 mM glutamine (Sigma Chemicals Co., St Louis, MO, USA), 75 µg/mL streptomycin (Sigma Chemicals Co., St Louis, MO, USA), and 100 µg/mL penicillin (Sigma Chemicals Co., Chan-

tilly, VA, USA) for 24 h. Conditioned media from all cultures were collected and stored for cytokine determinations.

Quantification of cytokines

Cytokines were measured in lung tissue homogenates by a specific two-site sandwich ELISA, using the following mAb: for IL-4, BVD-1D11 and biotinylated BVD6-24G2; for IL-5, TRFK5 and biotinylated TRFK4; and for IFN- γ , XMG 1.2 and biotinylated AN18. Binding of biotinylated mAbs detected using a streptoavidin-horseradish peroxidase complex (Amersham Int., Amersham, UK) and 2-2'-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) (Sigma) in 0.1 M citrate buffer containing hydrogen peroxide. The detection limits were 7.8 pg/mL for IL-4; 15.6 pg/mL for IL-5; 31.3 pg/mL for IL-10; 0.31 pg/mL for IL-13; 31.3 pg/mL for IFN- γ ; and 500 pg/mL for eotaxin.

Fibronectin assay

The concentrations of fibronectin present in lung tissue homogenates were determined using a competitive ELISA method [31]. In brief, 96-well plates (Fisher Scientific, Pittsburgh, PA, USA) were coated overnight with plasma fibronectin at 1 µg/mL in carbonate buffer, pH 9.6. In a separate 96-well plate, samples and several concentrations of a standard fibronectin solution were incubated with polyclonal anti-fibronectin rabbit antiserum overnight at 4°C . Plates coated with fibronectin were blocked with 1% bovine serum albumin in TBS at room temperature for 30 min. After rinsing three times with TBS+0.05% Tween-20, 100 µL of antigen-antibody incubations were added to plates and incubated for 2 h at room temperature. The wells were washed, and the free antibody bound to the plate was detected using biotinylated goat anti-rabbit antibody (Sigma Chemicals Co., St Louis, MO, USA). The reactions were developed with streptoavidin-peroxidase conjugate (Sigma Chemicals Co., St Louis, MO, USA), OPD, and H_2O_2 , and the plates were read at 450 nm on an automated ELISA reader (Dynatech, MR 5000, Chantilly, VA, USA). Samples were quantified by comparison with standard curves of recombinant mouse fibronectin. The minimal amount of fibronectin that could be detected was 3 ng/mL.

Histology of the lungs

Histology was performed as described elsewhere [32]. Paraffin-embedded sections (5 µm) were stained with haematoxylin/eosin (H&E) to evaluate general morphology, and goblet cells were visualized on periodic acid-Schiff (PAS)-stained lung sections. Collagen deposition was on Picro Sirius red-stained sections. All slides were examined with light microscopy at a magnification of $\times 40$. For each group of six mice, four stained lung sections from each mouse were analysed. Scale bar: 15 µm.

Quantitation of lung oedema

As a separate measure of pulmonary oedema formation, wet-to-dry lung weight ratios were determined for both the left and right lungs. Six and 24 h after last aerosol challenge, the lungs of groups of mice were excised separately, and each lung was rapidly weighed on pre-tared dishes for the determination of wet lung weight. The samples were then dried in an oven (Fisher isotemp, 65 °C) for 3–4 days and weighed daily to establish dry lung weight. The wet-to-dry lung weight ratio is expected to increase with an increase in extra vascular lung water, blood volume, or both.

Statistical analysis

All values were expressed as mean $\pm$ SEM. Parametric data were evaluated using analysis of variance, followed by the Tukey test for multiple comparisons. Non-parametric data were assessed using the Mann–Whitney test. Differences were considered to be statistically significant at $P < 0.05$. The SPSS statistical package (Release 13.0, Evaluation Version, 2004) was used.

Results

Administration of methysergide reduces sustained airway inflammation induced by prolonged ovalbumin challenge

To evaluate the role of serotonin in the inflammatory and remodelling processes that follow a prolonged allergen challenge, the effects of the 5-HT₂ receptor antagonist methysergide (40 μ g/kg, i.n., 2 h before each challenge) were determined. BAL of OVA/PBS mice contained only a few macrophages and no eosinophils or neutrophils were noted. Prolonged OVA challenge of vehicle-treated mice (OVA/OVA) induced a large number of eosinophils in BAL. Compared with the vehicle-treated OVA/OVA group, methysergide-treated mice immunized and challenged with OVA showed absence of eosinophil or neutrophils (Fig. 1a). In addition, lung wet-to-dry weight ratio analysis demonstrated a significantly lower water content in the lung of methysergide-treated mice than in the vehicle-treated OVA/OVA mice, 6 and 24 h after the last aerosol challenge (Fig. 1b). We further analysed the fibronectin level by an immunoenzymatic assay. A significant reduction in fibronectin content was noted in BAL from sensitized mice treated with methysergide (Fig. 2).

IL-4 and IL-5 are the main cytokines involved in allergic airway inflammation and, therefore, we measured their levels in explants of lung tissue after challenge with aerosolized OVA in vehicle- or methysergide-treated OVA/OVA mice. The choice of the lung for the cytokines evaluation is based on the fact that bronchial intraepithelial CD4 and CD8 allergen-specific T lymphocytes seem to be more responsible for the production of the proinflammatory

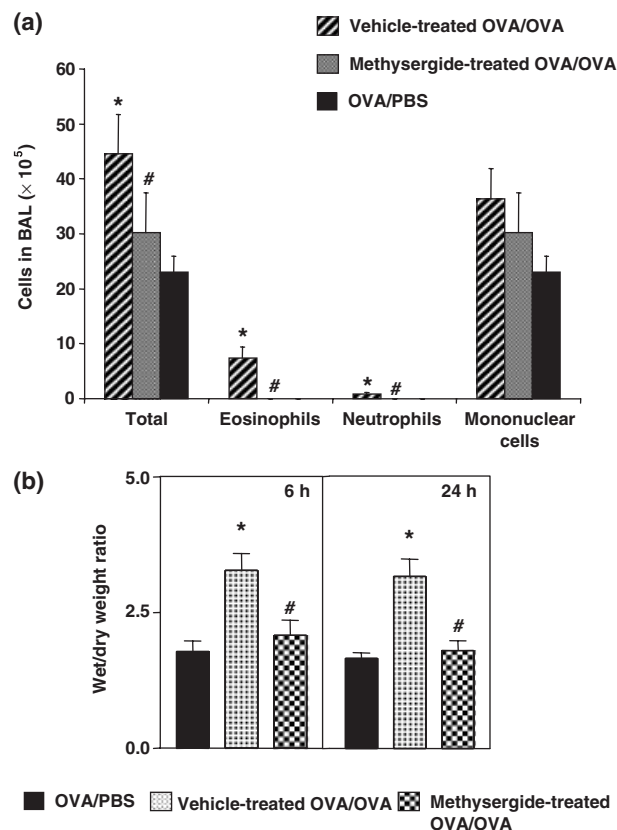


Fig. 1. Effects of methysergide on bronchoalveolar lavage (BAL) eosinophilia. Immunized mice were exposed to aerosolized ovalbumin (OVA) for 20 min twice a week, for 4 consecutive weeks. Two hours before each challenge, they were treated with vehicle (saline, 40 μ L intra nasally) or methysergide (i.n., 40 μ g/kg). Forty-eight hours after the last aerosol challenge, BAL was collected for cell counts ($\times 10^5$), (a) and wet-to-dry lung weight ratios were determined for both the left and right lungs (b). The results represent the mean $\pm$ SEM of six animals/group. * $P < 0.001$ compared with the OVA/phosphate-buffered saline (PBS) group; # $P < 0.01$ compared with the vehicle-treated OVA/OVA group.

cytokines associated with inflammation and asthma, different from the circulating lymphocytes in the blood or present in BAL [33]. Figures 3a and b show that both cytokines were detected in the vehicle-treated OVA/OVA group. However, in methysergide-treated mice, the amounts of T helper type 2 (Th2) cytokines (IL-4 and IL-5) in lung explants were significantly reduced as compared with those from vehicle-treated OVA/OVA mice. Interestingly, the administration of methysergide significantly increased the amount of IFN- γ released by the explants from lungs provided by mice following a challenge with OVA (Fig. 3c).

Methysergide suppresses ovalbumin-specific responses in vitro

OVA-specific immune responses in spleen and TLN cultures were performed to assess whether methysergide

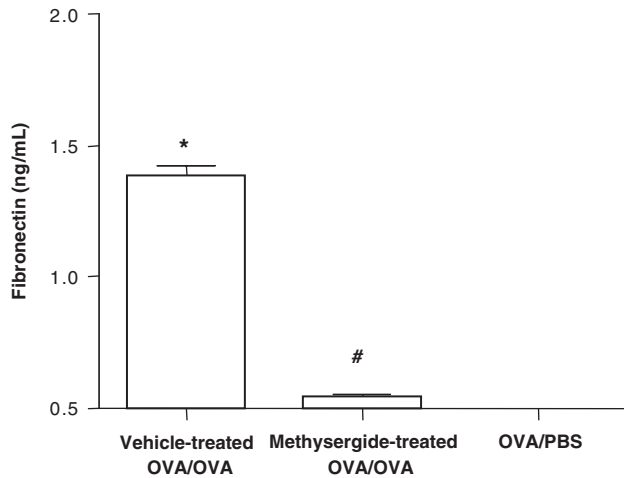


Fig. 2. Effects of methysergide on fibronectin production. Forty-eight hours after the last aerosol challenge, bronchoalveolar lavage was collected for fibronectin determination by ELISA. Each column indicates the mean $\pm$ SEM of six animals/group. The minimal amount of fibronectin that could be detected was 3 ng/mL. * $P < 0.001$ compared with the ovalbumin (OVA)/phosphate-buffered saline (PBS) group; # $P < 0.01$ compared with the vehicle-treated OVA/OVA group.

treatment directly affected lymphocyte function. The OVA-specific production of IL-4, IL-5, and Eotaxin was significantly decreased in both the spleen and TLN of mice treated with methysergide. In these mice, the levels of IL-13 or IFN- γ were only decreased in the spleen or TLN, respectively. Mice treated with methysergide showed no significant difference in OVA-induced IL-10 levels in spleen or lymph node cultures (Fig. 4).

Inhibition of airway remodelling does not correlate with plasma levels of interferon- γ

We studied whether the protection of mice against airway chronic inflammation and remodelling by the administration of methysergide resulted from the presence of IFN- γ in their lung. When methysergide-treated mice were exposed to IFN- γ -blocking antibodies and subsequently submitted to OVA, inhibition of eosinophil recruitment into the BAL persisted (Fig. 5a). A significant increase in the level of IgE was found after OVA challenge of vehicle-treated animals (OVA/OVA). Methysergide-treated mice injected with control antibodies had less plasma IgE than vehicle-treated OVA/OVA, and plasma from the methysergide group treated with IFN- γ -blocking antibodies had even less (Fig. 5b). The anti-IFN- γ antibodies failed to prevent the inhibitory effect of methysergide on the elevated levels of Th2 cytokines in lung homogenates (Fig. 6).

Sub-epithelial matrix collagen deposition is characteristic of the remodelled airway. Prolonged allergen challenge of sensitized mice resulted in increased fibronectin levels in BAL (Fig. 2) and in homogenates of lung tissue

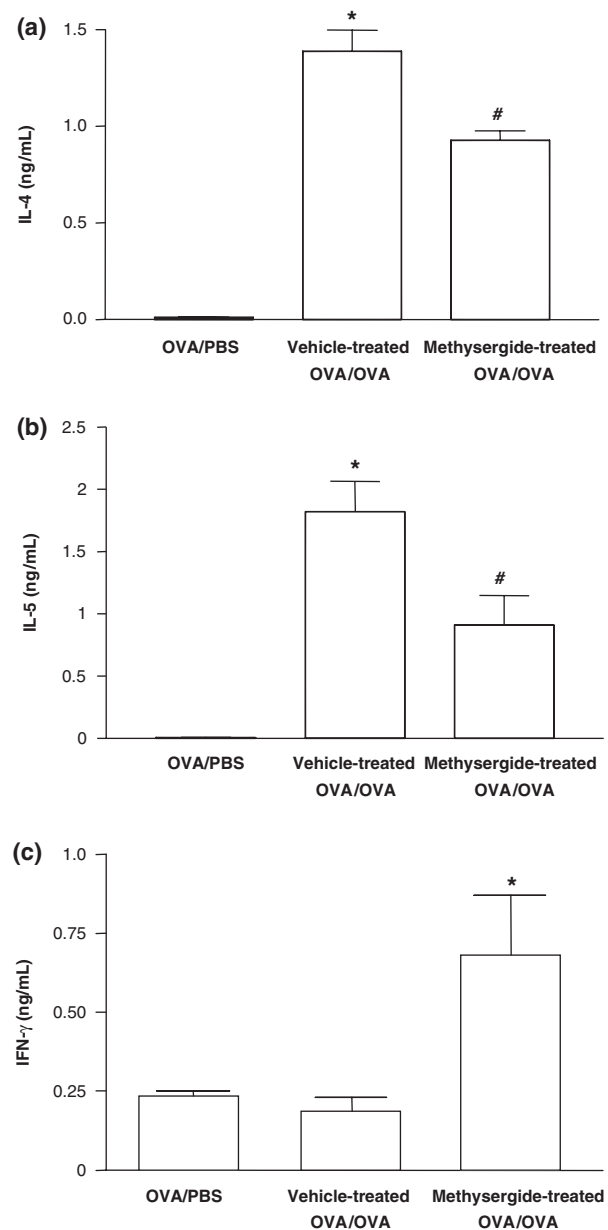


Fig. 3. Modulation of cytokine production by methysergide. Levels of IL-4 (a), IL-5 (b), and IFN- γ (c) were measured in conditioned media from lung explants by ELISA in mice treated with vehicle (saline, 40 μ L intra nasally) or methysergide during prolonged allergen challenge. Data are expressed as mean $\pm$ SEM of 6 animals/group. Detection limits were 7.8 pg/mL for IL-4, 15.6 pg/mL for IL-5, and 31.3 pg/mL for IFN- γ . * $P < 0.001$ compared with ovalbumin (OVA)/phosphate-buffered saline (PBS) group; # $P < 0.01$ compared with the vehicle-treated the OVA/OVA group.

(Fig. 7a) and in collagen deposition in the airways (Fig. 7b). Collagen deposition was substantially reduced in lungs from mice treated with methysergide as compared with vehicle-treated OVA/OVA mice. Further, the treatment of these mice with IFN- γ blocking antibodies failed to restore the levels of fibronectin or collagen deposition in lung tissue (Figs 7a and b).

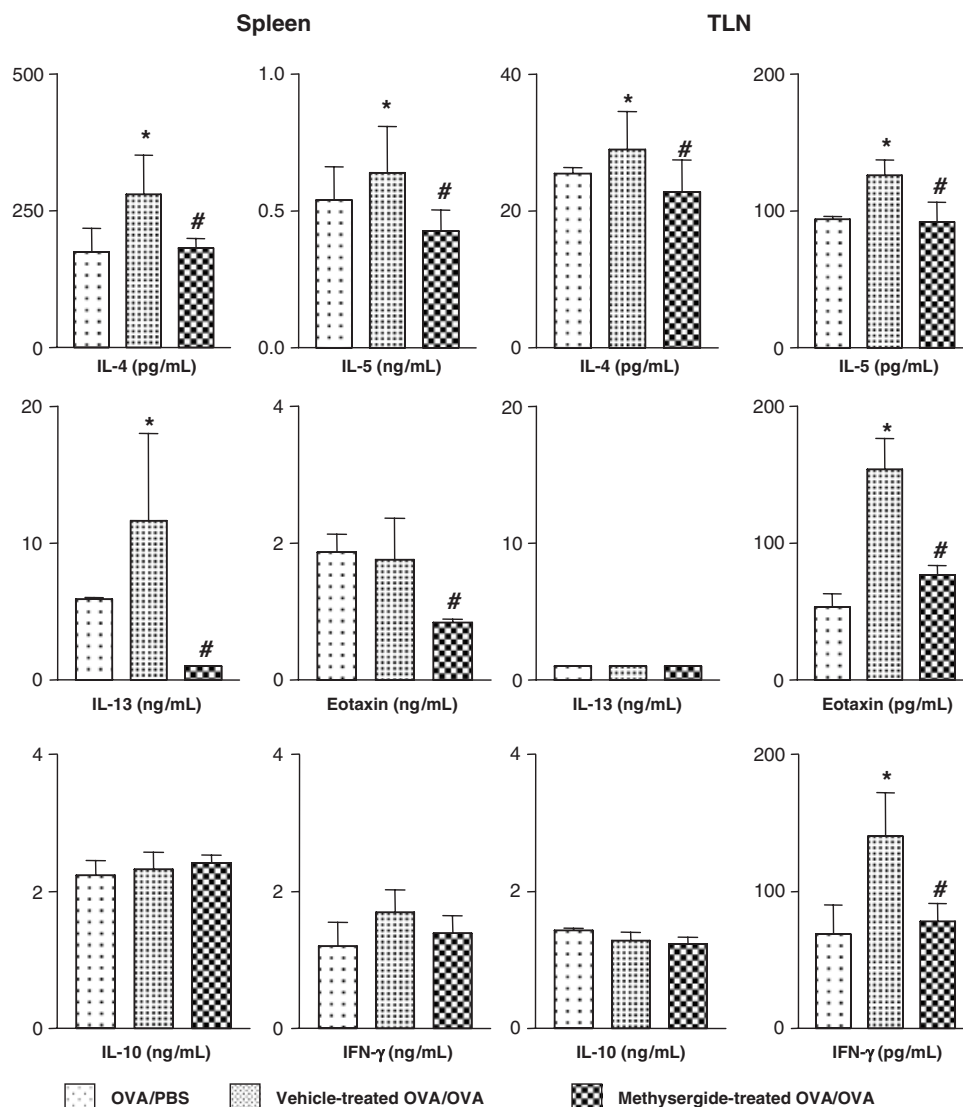


Fig. 4. Reduced *in vitro* ovalbumin (OVA)-specific responses in mice treated with methysergide. Spleen and thoracic lymph node cells ($n = 6$ mice/group) were harvested from mice on day 38 and cultured for 72 h with OVA (500 $\mu\text{g/mL}$). IL-4, IL-5, IL-13, eotaxin, IL-10, or IFN- γ concentrations in the culture supernatants were measured by ELISA. Data are expressed as the mean $\pm$ SEM ($n = 6$). * $P < 0.05$ compared with the OVA/phosphate-buffered saline (PBS) group; # $P < 0.05$ compared with the vehicle-treated OVA/OVA group.

Effect of serotonin on airway chronic inflammation

Figure 8a shows that prolonged challenge of OVA-immunized mice with i.n. serotonin induced a similar influx of eosinophils into the BAL as compared with vehicle-treated OVA/OVA mice. PAS-positive cells were not observed in the bronchial epithelium after prolonged serotonin challenge, but only in animals exposed to repeated OVA challenge (Fig. 8b). Eosinophil influx was associated with a significant increase in IL-4 and IL-5 levels in lung homogenates (Fig. 9). Interestingly, after prolonged serotonin challenge, an increase in collagen deposition was observed in the sub-epithelial layer of the airways, and an increased fibronectin level in lung tissue (Fig. 10). Instilla-

tion of serotonin in non-immunized mice did not induce eosinophil recruitment to BAL, Th2 cytokine production nor fibronectin release and collagen deposition (data not shown).

Discussion

Most studies of 'asthma models' in mice have used relatively short-term protocols for antigen sensitization and challenge, which can replicate some but not all of the features of chronic asthma in humans. The long-term changes in the airways of chronic asthma called airway remodelling are characterized by a persistent airway

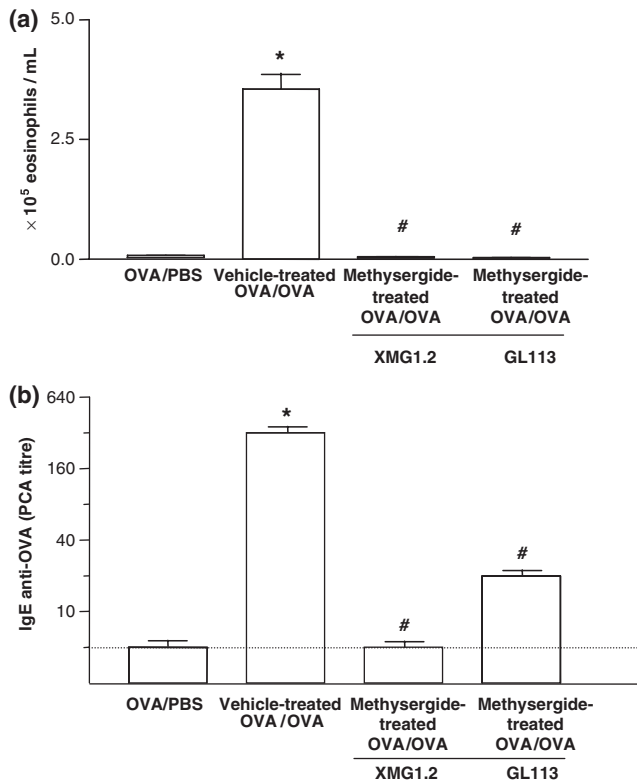


Fig. 5. Effect of IFN- γ neutralization on eosinophilia and specific IgE titres. Two hours before each challenge, mice were treated with vehicle (saline, 40 μ L intra nasally) or methysergide, and 1 h before with rat anti-mouse IFN- γ mAb (XMG 1.2) or isotype control MoAb (GL113). Eosinophil counts in bronchoalveolar lavage ($\times 10^5$) were obtained 48 h after the last aerosol challenge (a) Bars represent the mean $\pm$ SEM of 6 animals/group. Mice were bled for IgE determination by passive cutaneous anaphylaxis (PCA) (b) The PCA titre represents the highest dilution of pooled plasma that gave a positive reaction (diameter > 5 mm). The dashed line represents the detection threshold. * $P < 0.01$ compared with the ovalbumin (OVA)/phosphate buffer saline (PBS) group; # $P < 0.01$ compared with the vehicle-treated OVA/OVA group.

inflammation, increased number of mucus-secreting goblet cells in the airway epithelium with excessive mucus production, and increased deposition of collagen in the lungs, smooth muscle cell hyperplasia/hypertrophy, as well as airway hyperresponsiveness [10, 34, 35]. In the present study, we found that BALB/c mice exposed to prolonged allergen challenge developed significant airway remodelling (deposition of interstitial collagens and augmented fibronectin production) in association with an increase in airway eosinophilic inflammation with IL-4, IL-5, IL-13, and eotaxin production, protein exudation, specific IgE secretion, and goblet cell hyperplasia.

Rodents have large amounts of serotonin (5-HT) in the granules of their mast cells. Serotonin is secreted abundantly during the allergic response and its relevance for acute bronchoconstriction seems clear [13–15]. We investigated the effect of serotonin released after IgE-mediated

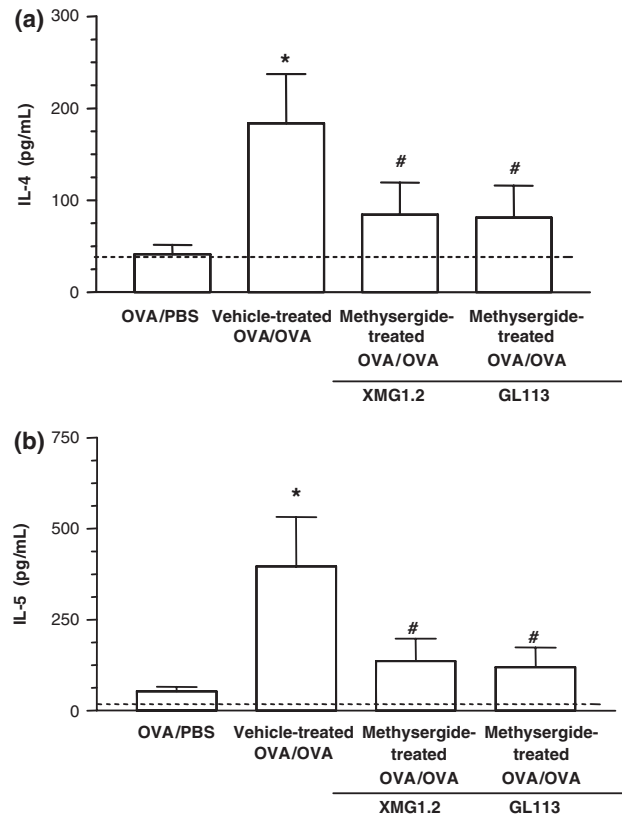


Fig. 6. Effect of IFN- γ neutralization on T helper type 2 cytokines. Levels of IL-4 (a) and IL-5 (b) were measured in homogenate of lung tissue of mice treated with vehicle (saline, 40 μ L intra nasally) or methysergide and with rat anti-mouse IFN- γ mAb (XMG 1.2) or isotype control MoAb (GL113) before each challenge. The detection limit was 7.8 pg/mL for IL-4 or 15.6 pg/mL for IL-5. * $P < 0.01$ compared with the ovalbumin (OVA)/phosphate-buffered saline group; # $P < 0.05$ compared with the vehicle-treated OVA/OVA group.

mast cell degranulation on the remodelling process using of methysergide, a specific 5-HT receptor antagonist.

Methysergide, instilled i.n. 2 h before each repeated challenge, inhibited the allergen-induced increase in airway eosinophilia, goblet cell hyperplasia, and significantly reduced specific IgE levels. The cytokines IL-4, IL-5, IL-13, and eotaxin produced by bronchial intraepithelial allergen-reactive CD4⁺ Th2 cells and locally by inflammatory cells recruited into the airways are responsible for many features of allergic asthma including specific IgE production, eosinophilia, and mucus hypersecretion [36]. The observed reduction in pulmonary inflammation, goblet cell hyperplasia, and specific IgE synthesis might have resulted from the reduction in the lung tissue levels of IL-4 and IL-5 following treatment with methysergide. In addition, the anti-inflammatory effect of methysergide is at least in part mediated through a suppressive action on T lymphocytes, as OVA-specific IL-4, IL-5, IL-13, and eotaxin secretions were reduced in spleen and thoracic lymph node cultures from mice

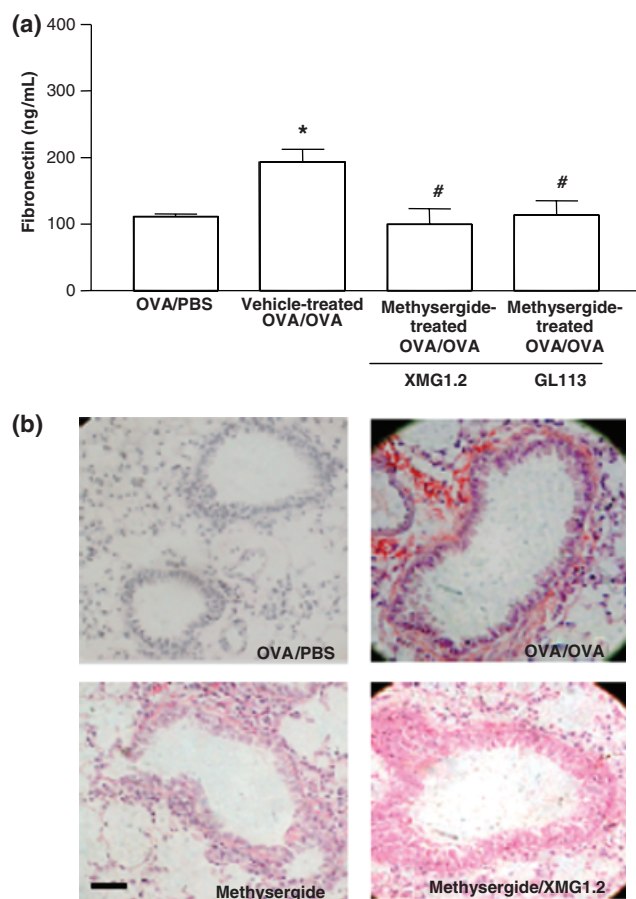


Fig. 7. Effect of IFN- γ neutralization on airway remodelling. Ovalbumin (OVA)-immunized mice were exposed to aerosolized OVA for 20 min twice a week for four consecutive weeks. Two hours before each challenge they were treated with vehicle (saline, 40 μ L intranasally) or methysergide, and 1 h with rat anti-mouse IFN- γ mAb (XMG 1.2) or isotype control MoAb (GL113). Lungs were obtained 48 h after the last aerosol challenge, for fibronectin determination (a) and for Picro Sirius red staining at $\times 40$ magnification (b). * $P < 0.05$ compared with the OVA/phosphate buffer saline (PBS) group; # $P < 0.05$ compared with the vehicle-treated OVA/OVA group. Scale bar: 15 μ m.

treated with methysergide. The reduction in Th2 cytokine production in both lymphoid organ cultures was not accompanied by an increase in the secretion of IFN- γ , a Th1 cytokine or IL-10, an anti-inflammatory cytokine. Indeed, IFN- γ production was also reduced in lymph node cultures.

In contrast to methysergide's effect on T lymphocytes in lymphoid organs, the treatment of mice with methysergide up-regulated the lung levels of IFN- γ , suggesting that the suppression of the allergic parameters and the protection against airway remodelling might have resulted from the presence of IFN- γ in the effector organ (lung compartment). This nevertheless seems unlikely, as neutralizing anti-IFN- γ antibodies administered to mice after methysergide instillation and before OVA challenge did not prevent the inhibitory effect of methysergide on Th2

inflammation or on airway remodelling in the lung. IFN- γ suppresses proliferation of Th2 cells [37], inhibits IgE production [38], and promotes Th1 differentiation by inducing IL-12 receptor β_2 -chain expression on T cells [39]. In addition, when administered before inhaled antigen challenge, IFN- γ reduces the number of CD4⁺ T cells in the respiratory tract and consequently eosinophil recruitment into the mouse airways [40–42]. In contrast, there is now evidence that Th1 cells [43, 44] and IFN- γ [45] secretion may exacerbate airway inflammation in asthma.

The mechanisms that increase the permeability of the airway epithelium and of the arterial endothelium in asthma are not yet completely understood. However, plasma exudation in the airway interstitium and lumen caused by an increase in the endothelial and epithelial permeability might be due to the release of inflammatory mediators, which in turn leads to microvascular and epithelial leakage [46, 47]. Microvascular permeability plays a key role in airway narrowing contributing to abnormal airway function in chronic asthma [6, 48]. As demonstrated clearly, serotonin increases vascular permeability via stimulation of 5-HT_{2B} receptors in lung endothelial cells [49]. Our data show that methysergide abrogated the extravasation of plasma from the vascular into the alveolar space in the lung of treated compared with untreated OVA/OVA mice.

Previous studies examining increased sub-epithelial extracellular matrix deposition in murine models of airway remodelling have measured changes in collagen [50–52] and fibronectin [34, 53]. In this regard, administration of methysergide resulted in the abrogation of airway fibronectin content, and collagen depositions in the lung were virtually absent in treated mice, suggesting the participation of serotonin in development of airway remodelling.

It is also known that mast cells play a pivotal role in the development of multiple features of asthma by producing cytokines, such as IL-4, IL-5, IL-6, IL-10, and IL-13 and TNF- α , which are important in the development and maintenance of Th2 allergic response [54–57]. Some *in vitro* studies have demonstrated that mast cells produce various mediators such as TGF- β 1 [58] and matrix metalloproteinases (MMPs) [59], which may contribute to the development of airway remodelling. However, Masuda et al. [60] demonstrated in mast cell-deficient mice immunized with OVA plus Al(OH)₃ and exposed to repeated OVA challenge that mast cells play a partial role in the development of subepithelial fibrosis, although antigen-induced airway inflammation and airway hyperreactivity are independent of mast cells. In addition, Yu et al. [61], working with mast cell knock-in mice sensitized with a repetitive i.n. challenge without adjuvant engrafted with FcR $\gamma^{+/+}$ (WT) vs. FcR $\gamma^{-/-}$ mast cells showed that mast cells, were essential for the full development of each

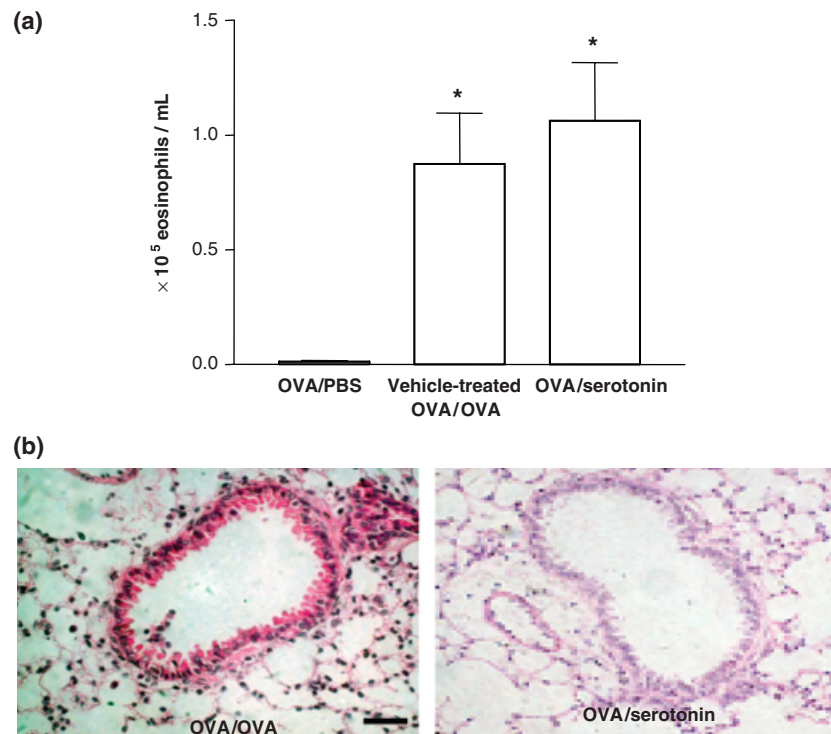


Fig. 8. Effect of serotonin on airway chronic inflammation. Mice immunized with ovalbumin (OVA) were exposed to aerosolized OVA or intranasal instillation of serotonin (1 µg/mL, 40 µL) two days a week, for four consecutive weeks ($n = 6$ /group). Twenty four hours after OVA or serotonin, killed mice were sacrificed and bronchoalveolar lavage was obtained for eosinophil counts ($\times 10^5$), (a) or lung tissue for periodic acid-Schiff staining (b) Stained lung sections were examined at $\times 40$ magnification. * $P < 0.01$ compared with the OVA/PBS group. Scale bar: 15 µm.

of the features of the chronic asthma model as increased number of lung mast cells, enhanced airway responsiveness to methacholine challenge, airway infiltrates of eosinophils and lymphocytes, epithelial goblet cell hyperplasia, and increased levels of lung collagen. Therefore, suitable animal models are needed to enable us to address properly the real role of mast cells and mast cell-derived mediators in the development of multiple features of chronic asthma.

Mast cells, but not T cells or eosinophils, localize near the airway smooth muscle (ASM) bundles in patients with asthma, but not in normal individuals or in those with eosinophilic bronchitis [62]. Smooth muscle mast cell density correlates significantly with BHR, and is likely to be an important factor determining the asthmatic phenotype. Interaction between ASM cells and mast cells, probably due to the enhanced release of mast cell growth factors, favours mast cell degranulation. Serotonin released from mast cells [63] upon activation by antigen-specific IgE in mice might function as a potent pulmonary vasoconstrictor [19] and can influence the development of BHR [13–15]. However, its role in the remodelling process following prolonged allergen exposure remains to be elucidated.

To confirm our hypothesis of the involvement of serotonin in remodelling, we reproduced the chronic model of airway inflammation. In agreement with our

results with OVA challenge, increased local serotonin levels in OVA-sensitized mice by repeated nasal instillation induced infiltration of inflammatory cells that were predominantly eosinophilic, and was accompanied by up-regulated levels of IL-4 and IL-5. The production of fibronectin and the deposition of collagen around the airways of mice challenged with serotonin were also observed as compared with OVA-challenged mice. In contrast, the hyperplasia of mucus cells was not observed in serotonin-instilled mice. With regard to this, Cohn et al. [45] showed that goblet cell differentiation does not occur in immune responses, particularly those rich in IFN- γ . In our model, we suggest that the absence of goblet cells induced by serotonin instillation could be the result of the presence of significant levels of IFN- γ in the lung.

An explanation for the observed effects of methysergide on airway inflammation and lung remodelling could be that binding of serotonin onto cognate receptors distributed throughout the airways or its cellular internalization [19] triggers the release of cytokines and of fibrogenic mediators that contribute to the development and maintenance of remodelling. In a recent work, Little et al. [26] confirmed the involvement of serotonin in the *in vivo* release of IL-16, a very potent chemoattractant for human eosinophils [64] and CD4⁺ T cells [26, 65]. These authors demonstrated that allergen stimulation of bronchial epithelial cells results in the production and

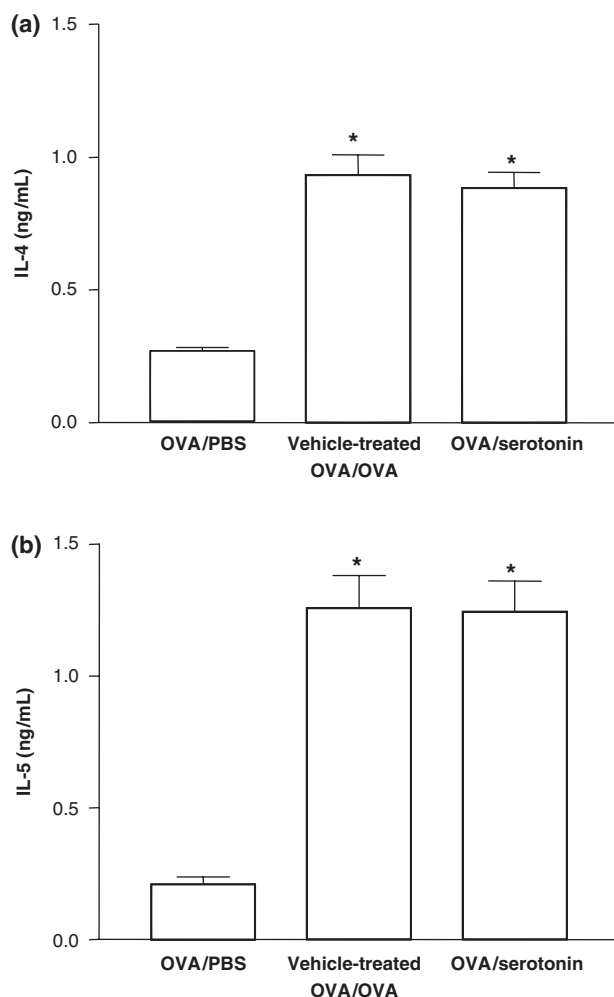


Fig. 9. Serotonin stimulated T helper type 2 cytokines in the lung. Levels of IL-4 (a) and IL-5 (b) were measured in lung tissue homogenates by ELISA. Data are expressed as mean $\pm$ SEM of six animals/group. The detection limits were 7.8 pg/mL for IL-4 and 15.6 pg/mL for IL-5. * $P < 0.001$ compared with the OVA/phosphate buffer saline (PBS) group.

secretion of IL-16 only after serotonin stimulation. A recent study demonstrated that serotonin independently acts as a chemoattractant for eosinophils and this effect appears to be mediated by the interactions with the 5-HT_{2A} receptor expressed by eosinophils. Additionally, serotonin has an additive effect with eotaxin, inducing eosinophil chemotaxis and pulmonary eosinophilia [66].

Thus, in our model, the recruitment of eosinophils and the activation of CD4⁺ Th2 lymphocytes and subsequent secretion of IL-4, IL-5, IL-13, and eotaxin might be dependent on the release of IL-16 from bronchial epithelial cells after serotonin release dependent on mast cell activation. Moreover, the direct effect of serotonin on the proliferation and activation of CD4⁺ T helper cells has been described [21, 67]. In addition, evidences recently provided by O'Connell et al. [68] indicate that DCs can

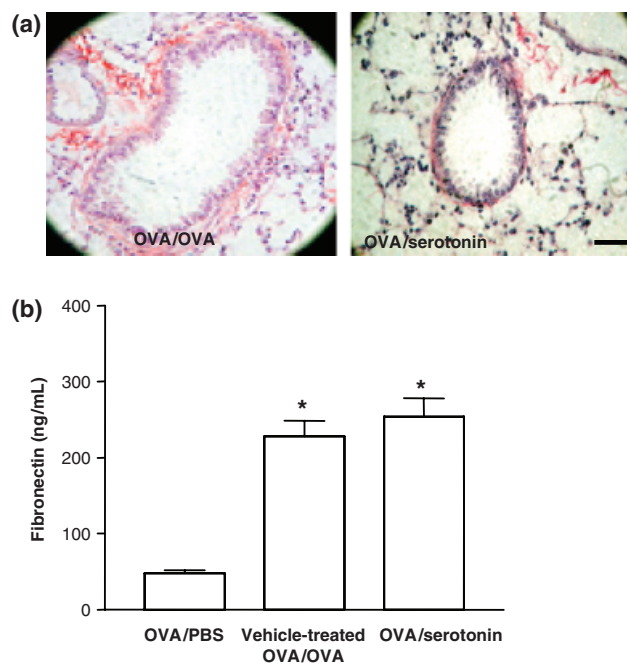


Fig. 10. Effect of serotonin on airway remodelling. Twenty four hours after ovalbumin (OVA) or serotonin challenge, mice were killed and lung tissues were obtained for Picro Sirius red staining (a) and for fibronectin determination (b). Stained lung sections were examined at $\times 40$ magnification. * $P < 0.05$ compared with the OVA/phosphate buffer saline (PBS) group. Scale bar: 15 μ m.

regulate T cell proliferation and differentiation via serotonin. Resident DCs in peripheral tissues, including the skin and lung, may uptake serotonin that is released by activated platelets, degranulating mast cells, or sympathetic nerves. This stored serotonin is then released via Ca²⁺-triggered exocytosis and can alter cAMP levels in T cells by acting at type 1 5-HT receptors.

Overall, our data indicate that serotonin release dependent on mast cell activation may contribute to the development and maintenance of remodelling, through action on eosinophils, and the induction of cytokines and fibrogenic mediator release. Serotonin should therefore be considered as a relevant signalling for the development and maintenance of airway remodelling.

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References

- 1 Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; 1:520–4.
- 2 Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990; 3:507–11.

- 3 Roberts CR. Is asthma a fibrotic disease? *Chest* 1995; 107:111S-7S.
- 4 Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. *Clin Exp Allergy* 1997; 27:363-71.
- 5 Chu HW, Halliday JL, Martin RJ, Leung DY, Szefer SJ, Wenzel SE. Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. *Am J Respir Crit Care Med* 1998; 158:1936-44.
- 6 James AL, Paré PD, Hogg JC. The mechanisms of airway narrowing in asthma. *Am Rev Respir Dis* 1989; 139:242-6.
- 7 Kay AB, Phipps S, Robinson DS. A role for eosinophils in airway remodelling in asthma. *Trends Immunol* 2004; 25:477-82.
- 8 Humbles AA, Lloyd CM, Mcmillan SJ *et al.* A critical role for eosinophils in allergic airways remodeling. *Science* 2004; 305:1776-9.
- 9 Flood-Page P, Menzies-Gow A, Phipps S *et al.* Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003; 112:1029-36.
- 10 McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 2004; 34:497-507.
- 11 Snibson KJ, Bischof RJ, Slocombe RF, Meeusen EN. Airway remodelling and inflammation in sheep lungs after chronic airway challenge with house dust mite. *Clin Exp Allergy* 2005; 35:146-52.
- 12 Dvorak AM, Tepper RI, Weller PF *et al.* 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* 1994; 83:3600-12.
- 13 Nagai H, Yamaguchi S, Maeda Y, Tanaka H. Role of mast cells, eosinophils and IL-5 in the development of airway hyperresponsiveness in sensitized mice. *Clin Exp Allergy* 1996; 26:642-7.
- 14 Matkar NM, Rupwate RU, Desai NK, Kamat SR. Comparative study of platelet histamine and serotonin with their corresponding plasma oxidases in asthmatics with normals. *J Assoc Physicians India* 1999; 47:878-82.
- 15 Eum SY, Norel X, Lefort J, Labat C, Vargaftig BB, Brink C. Anaphylactic bronchoconstriction in BP2 mice: interactions between serotonin and acetylcholine. *Br J Pharmacol* 1999; 126:312-6.
- 16 Idzko M, Panther E, Stratz C *et al.* The serotonergic receptors of human dendritic cells: identification and coupling to cytokine release. *J Immunol* 2004; 172:6011-9.
- 17 Hoyer D, Clarke DE, Fozard JR *et al.* International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 1994; 46:157-203.
- 18 Ullmer C, Schmuck K, Kalkman HO, Lubbert H. Expression of serotonin receptor mRNAs in blood vessels. *FEBS Lett* 1995; 370:215-21.
- 19 MacLean MR, Herve P, Eddahibi S, Adnot S. 5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension. *Br J Pharmacol* 2000; 131:161-8.
- 20 Bayer H, Muller T, Myrtek D *et al.* Serotonergic receptors on human airway epithelial cells. *Am J Respir Cell Mol Biol* 2007; 36:85-93.
- 21 Young MR, Kut JL, Coogan MP, Wright MA, Young ME, Matthews J. Stimulation of splenic T-lymphocyte function by endogenous serotonin and by low-dose exogenous serotonin. *Immunology* 1993; 80:395-400.
- 22 Iken K, Cheng S, Fargin A, Goulet AC, Kouassi E. Serotonin upregulates mitogen-stimulated B lymphocyte proliferation through 5-HT_{1A} receptors. *Cell Immunol* 1995; 163:1-9.
- 23 Laberge S, Cruikshank WW, Beer DJ, Center DM. Secretion of IL-16 (lymphocyte chemoattractant factor) from serotonin-stimulated CD8+T cells *in vitro*. *J Immunol* 1996; 156:310-5.
- 24 Cloez-Tayarani I, Petit-Bertron AF, Venters HD, Cavaillon JM. Differential effect of serotonin on cytokine production in lipopolysaccharide-stimulated human peripheral blood mononuclear cells: involvement of 5-hydroxytryptamine_{2A} receptors. *Int Immunol* 2003; 15:233-40.
- 25 Lima C, Souza VMO, Faquim-Mauro EL *et al.* Modulation of the induction of lung and airway allergy in the offspring of IFN- γ -treated mother mice. *J Immunol* 2005; 175:3554-9.
- 26 Little FF, Lynch E, Fine G, Center DM, Cruikshank WW. Tumor necrosis factor- α -induced synthesis of interleukin-16 in airway epithelial cells: priming for serotonin stimulation. *Am J Respir Cell Mol Biol* 2003; 28:354-62.
- 27 Fozard JR, Leach GDH. The hypotensive action of 5-hydroxytryptamine (5-HT) in anaesthetised and pithed rats. *Eur J Pharmacol* 1968; 2:239-49.
- 28 Hannon JP, Tigani B, Williams I, Mazzoni L, Fozard JR. Mechanism of airway hyperresponsiveness to adenosine induced by allergen challenge in actively sensitized Brown Norway rats. *Br J Pharmacol* 2001; 132:1509-23.
- 29 Mota I, Wong D. Homologous passive cutaneous anaphylactic activity of mouse antiserum during the course of immunization. *Life Sci* 1969; 8:813-20.
- 30 Proust B, Nahori MA, Ruffie C, Lefort J, Vargaftig BB. Persistence of bronchopulmonary hyper-reactivity and eosinophilic lung inflammation after anti-IL-5 or -IL-13 treatment in allergic BALB/c and IL-4 α knockout mice. *Clin Exp Allergy* 2003; 33:119-31.
- 31 Rennard SI, Berg R, Martin GR, Foidart JM, Robey PG. Enzyme-linked immunoassay (ELISA) for connective tissue components. *Anal Biochem* 1980; 104:205-14.
- 32 Reinhardt AK, Bottoms SE, Laurent GJ, McNulty RJ. Quantification of collagen and proteoglycan deposition in a murine model of airway remodelling. *Respir Res* 2005; 8:6-30.
- 33 Hodge G, Hodge S, Reynolds PN, Holmes M. Compartmentalization of intracellular proinflammatory cytokines in bronchial intraepithelial T cells of stable lung transplant patients. *Clin Exp Immunol* 2006; 145:413-9.
- 34 Trifilieff A, El Hashim A, Bertrand C. Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment. *Am J Physiol Lung Cell Mol Physiol* 2000; 279:L1120-8.
- 35 Komai M, Tanaka H, Masuda T *et al.* Role of Th2 responses in the development of allergen-induced airway remodelling in a murine model of allergic asthma. *Br J Pharmacol* 2003; 138:912-20.
- 36 Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM. Predominant Th2 like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298-304.
- 37 Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996; 383:787.

- 38 Finkelman FD, Katona IM, Mosmann TR, Coffman RL. Interferon- γ regulates the isotypes of immunoglobulin secreted during *in vivo* humoral immune responses. *J Immunol* 1988; **140**:1022.
- 39 Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the interleukin (IL)-12RB2 subunit expression in developing T helper 1 (Th1) and Th2 cells. *J Exp Med* 1997; **185**:817.
- 40 Iwamoto I, Nakajima H, Endo H, Yoshida S. Interferon gamma regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4+T cells. *J Exp Med* 1993; **177**:573.
- 41 Nakajima H, Iwamoto I, Yoshida S. Aerosolized recombinant interferon-gamma prevents antigen-induced eosinophil recruitment in mouse trachea. *Am Rev Respir Dis* 1993; **148**:1102.
- 42 Li XM, Chopra RK, Chou TY, Schofield BH, Wills-Karp M, Huang SK. Mucosal IFN-gamma gene transfer inhibits pulmonary allergic responses in mice. *J Immunol* 1996; **157**:3216.
- 43 Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyper-reactivity but cause severe airway inflammation. *J Clin Invest* 1999; **103**:175.
- 44 Randolph DA, Carruthers CJ, Szabo SJ, Murphy KM, Chaplin DD. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J Immunol* 1999; **162**:2375.
- 45 Cohn L, Homer RJ, Niu N, Bottomly K. T helper 1 cells and interferon gamma regulate allergic airway inflammation and mucus production. *J Exp Med* 1999; **190**:1309–18.
- 46 Laitinen A, Laitinen LA. Vascular beds in the airways of normal subjects and asthmatics. *Eur Respir J* 1990; **12**:658s–62s.
- 47 Regnard J, Lockhart A. Vascular factors in bronchial hyperresponsiveness and asthma. *Respir Med* 1992; **86**:179–81.
- 48 McFadden ER Jr, Gilbert IA. Asthma. *N Engl J Med* 1992; **327**:1928–37.
- 49 Launay JM, Herve P, Peoc'h K *et al*. Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat Med* 2002; **8**:1129–35.
- 50 Tang W, Geba GP, Zheng T *et al*. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. *J Clin Invest* 1996; **98**:2845–53.
- 51 Kenyon NJ, Ward RW, McGrew G, Last JA. TGF- β 1 causes airway fibrosis and increased collagen I and III mRNA in mice. *Thorax* 2003; **58**:772–7.
- 52 Cho JY, Miller M, Baek KJ *et al*. Inhibition of airway remodeling in IL-5-deficient mice. *J Clin Invest* 2004; **113**:551–60.
- 53 Trifilieff A, Fujitani Y, Coyle AJ, Kopf M, Bertrand C. IL-5 deficiency abolishes aspects of airway remodelling in a murine model of lung inflammation. *Clin Exp Allergy* 2001; **31**:934–42.
- 54 Dunford PJ, O'Donnell N, Riley JP, Williams KN, Karlsson L, Thurmond RL. The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of CD4+T cells. *J Immunol* 2006; **176**:7062–70.
- 55 Brightling CE, Bradding P, Pavord ID, Wardlaw AJ. New insights into the role of the mast cell in asthma. *Clin Exp Allergy* 2003; **33**:550–6.
- 56 Suto H Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* 2006; **176**:4102–12.
- 57 Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006; **117**:1277–84.
- 58 Kanbe N, Kurosawa M, Nagata H, Saito H, Miyachi T. Cord blood-derived cultured human mast cells produce transforming growth factor beta1. *Clin Exp Allergy* 1999; **29**:105–13.
- 59 Dahlen B, Shute J, Howarth P. Immunohistochemical localisation of the matrix metalloproteinases MMP-3 and MMP-9 within the airways in asthma. *Thorax* 1999; **54**:590–6.
- 60 Masuda T, Tanaka H, Komai M *et al*. Mast cells play a partial role in allergen-induced subepithelial fibrosis in a murine model of allergic asthma. *Clin Exp Allergy* 2003; **33**:705–13.
- 61 Yu M, Tsai M, Tam SY, Jones C, Zehnder J, Galli SJ. Mast cells can promote the development of multiple features of chronic asthma in mice. *J Clin Invest* 2006; **116**:1633–41.
- 62 Bradding P. The role of the mast cell in asthma: a reassessment. *Curr Opin Allergy Clin Immunol* 2003; **3**:45–50.
- 63 Kitamura Y. Heterogeneity of mast cells and phenotypic change between subpopulations. *Ann Rev Immunol* 1989; **7**:59–66.
- 64 Rand TH, Cruikshank WW, Center DM, Weller PF. CD4-mediated stimulation of human eosinophils: lymphocyte chemoattractant factor and other CD4-binding ligand elicit eosinophil migration. *J Exp Med* 1991; **173**:1521–8.
- 65 Center DM, Berman JS, Kornfeld H, Theodore AC, Cruikshank WW. The lymphocyte chemoattractant factor. *J Lab Clin Med* 1995; **125**:167–72.
- 66 Boehme SA, Lio FM, Sikora L *et al*. Cutting edge: serotonin is a chemotactic factor for eosinophils and functions additively with eotaxin. *J Immunol* 2004; **173**:3599–603.
- 67 Young MR, Matthews JP. Serotonin regulation of T-cell subpopulations and of macrophage accessory function. *Immunology* 1995; **84**:148–52.
- 68 O'Connell PJ, Wang X, Leon-Ponte M, Griffiths C, Pingle SC, Ahern GP. A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. *Blood* 2006; **107**:1010–7.