

IL-5 production by resident mucosal allergen-specific T cells in an explant model of allergic rhinitis

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Summary

Background Seasonal allergic rhinitis is a chronic inflammation in the nasal mucosa triggered by inhaled aeroallergens. The inflammatory reaction is controlled by allergen-specific T cells, but where and how these T cells become activated is not fully understood. **Objectives** We wanted to determine whether allergen-specific T-helper (Th) 2 cells are residing in the nasal mucosa under steady-state conditions outside of the pollen season and, if so, whether these cells are activated locally in response to allergen challenge.

Methods Mucosal biopsies from the lower turbinate were obtained out of season from patients with either birch- or grass-pollen-allergic rhinitis and from healthy controls.

Cultured explant samples were challenged with relevant pollen extract or with a mix of overlapping 20-mer peptides derived from the sequence of the major birch allergen, Betula verrucosa (Bet v) 1. After 24 h, culture medium was harvested for multiplex cytokine and tryptase analysis.

Results Significant amounts of interleukin (IL)-5 were secreted from resident cells in response to ex vivo allergen challenge in the allergic group only. No increase was observed for the other cytokines measured. Production of IL-5 in response to both extract and the Bet v1-derived peptide mix strongly suggested that T cells were a major source of IL-5.

Conclusion Our explant model indicated that local presentation of antigen to resident allergen-specific Th2 cells is the early event in the pathogenesis of allergic rhinitis. These findings identify possible cellular targets for anti-inflammatory treatment.

Keywords allergens, allergic disease, airway mucosa, T cells

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Introduction

Allergic rhinitis is a chronic inflammatory reaction in the airway mucosa triggered by aeroallergens which involves several types of immune cells. Allergic rhinitis represents a strong predictor of adult-onset asthma, and there is considerable evidence to support the hypothesis that there is a causal relationship between rhinitis and asthma [1]. Local allergen challenge of allergic rhinitis patients induces an acute reaction (within minutes), characterized by intense itching, sneezing and a runny nose, followed by a late-phase reaction (after hours) dominated by nasal blockage [2–4]. The early reaction is considered to be caused by histamine and other pro-

inflammatory molecules released by resident mast cells subsequent to IgE cross-linking, whereas the late-phase reaction is accompanied by accumulation of inflammatory cells in the nasal mucosa, primarily eosinophils and activated Th2 cells. The late-phase response has therefore been used as a model for the chronic allergic reaction. The relative contribution of mast-cell- vs. T-cell-dependent mechanisms in the pathogenesis of the allergen-induced late-phase response has not been determined, but several animal and human models for airway allergy have shown that allergen-specific Th2 cells are able to drive allergic inflammation in the airway mucosa independent of IgE-mediated mast-cell activation [5, 6]. In agreement with these results, several studies of allergic rhinitis have demonstrated an increased number of activated Th2 cells in the nasal

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mucosa 24 h after local allergen challenge [4, 7]. However, whether these activated Th2 cells were newly recruited cells or tissue-resident T cells that became activated locally has to our knowledge not been addressed. Allergen-specific T cells may be activated in organized lymphoid tissue draining the airways, with subsequent homing of post-activated T cells to the local site of challenge, or alternatively, they could be resident T cells activated locally. Where and how T cells involved in the allergic reaction are activated have important implications for therapeutic strategies.

To address this issue, we have established an explant model for allergic rhinitis. In this model, biopsy specimens from rhinitis patients obtained from the nasal mucosa out of season were cultured with or without allergen [8]. Our results indicated that local presentation of allergenic peptides to resident allergen-reactive Th2 cells could induce their activation and secretion of significant amounts of IL-5. The fact that T cells and antigen-presenting cells residing in the nasal mucosa under homeostatic conditions are able to drive an inflammatory reaction in response to allergen exposure independent of cell recruitment has implications for therapeutic strategies targeted at the inflammatory pathway.

Materials and methods

Participants

A total of 13 grass-pollen-allergic individuals, median age 24 years (range 22–34, seven women), 11 birch-pollen-allergic individuals, median age 28 years (range 12–50, seven women) and 16 non-allergic volunteers, median age 28 years (range 22–43, seven women) were included in this study. The participants were either skin prick tested with a panel of eight common inhalant allergens (house dust mite, mould, horse, dog, cat, grass, mugwort and birch) (Solu Prick SQ ALK-Abello A/S, Horsholm, Denmark, or Allergopharma, Reinbek, Germany) or tested for specific IgE against relevant inhalant allergens (ImmunoCAP[®], Thermofisher Scientific/Phadia, Uppsala, Sweden). Allergic individuals had a typical history of pollen-induced upper airway allergy and a positive skin prick test and/or specific IgE > 0.35 kU/L according to the European guidelines [9]. Twenty-one of the 24 allergic donors were multi-sensitized. Non-allergic volunteers had a history free of allergic symptoms and had a negative skin prick test to the same panel. Thirty of 40 participants were biopsied for research purposes only. They were all non-smoking; had no nasal polyps, nasal deformities or recent nasal surgery; had no allergy symptoms; and used no medication prior to or at the time of biopsy. Nasal mucosa from the remaining 10 individuals (two birch-allergic patients and eight controls) was obtained during sur-

gery for septum deviation. At time of surgery, they were all healthy and without any signs of nasal polyps or inflammation in the upper airways (patient description is found in the Table S1). For flow cytometric assessment of intracellular cytokine production, nasal mucosa was obtained from the lower edge of the inferior turbinate during surgery for septum deviation outside of the pollen season. The mucosa appeared macroscopically normal in all cases. The patients were selected based on whether they had a history of pollen allergies ($n = 6$) or not ($n = 5$). This study was approved by the Norwegian Regional Committee for Medical Research Ethics, and written consent was obtained from all participating subjects.

Flow cytometric analysis

Surgical specimens from pollen allergic and healthy donors were finely minced with scissors and incubated with 1 mg/mL Liberase TM (Roche, Mannheim, Germany). Identically treated PBMCs served as controls for the collagenase sensitivity of epitopes. To assess the cytokine production potential of mucosal T cells, dispersed cells were cultured for 5 h in RPMI/10% foetal calf serum (FCS) with 1.5 ng/mL phorbol 12-myristate 13-acetate (PMA) and 1 µg/mL ionomycin, with 10 µg/mL brefeldin A (all from Sigma-Aldrich, St Louis, MO, USA) added after 1 h of stimulation to allow intracellular accumulation of cytokines. Cells were stained with Fixable Viability Dye eFluor 450 (1 µL/10⁶ cells, eBioscience, San Diego, CA, USA) for 30 min at 4°C, followed by surface staining with 0.6 µg/mL CD3-BrilliantViolet 650 (clone OKT3), 5 µg/mL CD8-Alexa488 (clone SK1) and 1 µg/mL CD4-PE/Cy7 (clone SK3). To detect intracellular cytokines, cells were treated with Cytofix/Cytoperm according to the manufacturers protocol (BD Biosciences) and stained with 1 µg/mL IL-5-APC (clone TRFK5, Franklin Lakes, NJ, USA) and 1 µg/mL IL-13-PE (clone JES10-5A2). All antibodies were from Biolegend (San Diego, CA, USA) and were incubated for 30 min at 4°C. FACS was performed on a BD LSRFortessa (BD Biosciences) and analysed using FlowJo 7.6.3 software (Three Star, Ashland, OR, USA). The number of T cells acquired from each donor ranged between 4200 and 16 000, depending on the cellular yield from the surgical specimens. Gates for surface markers were set based on irrelevant isotype-matched antibodies, and cytokines gates was based on untreated cells.

Explant culture conditions

In experiments with grass-pollen extract, two biopsies were obtained from the lower edge of the inferior turbinate, 1.5–2 cm posterior to the front edge with a

Gerritsma forceps with a cup diameter of 2.5 mm. Local anaesthesia was applied by placing a cotton wool carrier with 7 mg/mL tetracaine/0.3 mg/mL adrenalin under the inferior turbinate. The samples were immediately placed on 0.4- μ m filter well inserts (Millicell Culture Plate Inserts, Millipore, Bedford, MA, USA) to form an air-liquid interface in six-well culture plates containing 2 mL bicarbonate-buffered culture medium (BCM). The culture medium was prepared as described previously [10] and consisted of Minimum Essential Medium (MEM; Life Technologies) supplemented with 2 mM L-glutamine (Lonza, Walkersville, MD, USA), 20 mL/L amino acid supplement (Life Technologies, Paisley, UK), 10 mL/L sodium pyruvate (Cambrex, Walkersville, MD, USA), 10 mL/L vitamin supplement (MEM Vitamins 100 $\times$, Life Technologies), 50 μ g/mL gentamycin (Cambrex, Walkersville, MD, USA), 0.1 μ g/mL insulin, 0.1 μ g/mL vitamin A and 0.1 μ g/mL hydrocortisone (all from Sigma-Aldrich). BCM was adjusted to pH 7.25 and sterile filtered. In experiments with birch-pollen extract, the biopsies were divided in two and cultured on filter well inserts in 24-well culture plates containing 400 μ L BCM to compensate for the smaller size of these tissue samples. Grass- and birch-pollen extracts (Aquagen timothy and Aquagen birch, ALK-Abello) were added to the culture medium at a final concentration of 60 μ g/mL and 100 μ g/mL, respectively. We also synthesized sixteen 20-mer peptides (overlapping with 10 amino acids) spanning the entire Bet v1 protein sequence (GL Biochem, Shanghai, China). A mix of all peptides was added to the culture medium at a final concentration of 10 μ M of each peptide. All specimens were cultured for 24 h in 5% CO₂/95% air. After culturing, the medium was collected and kept at -70°C, and the tissue specimens were embedded in OCT (Tissue-Tek) and snap-frozen in liquid nitrogen. To assess whether the 20-mer peptides have the potential to activate mast cells, a basophil activation assay was employed. To this end, PBMCs were obtained from birch-allergic donors and healthy controls ($n = 2$ in each group), and cells were enriched using a basophil isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified basophils were allowed to equilibrate at 37°C in RPMI/10% FCS, after which the cells were stimulated for 15 min with either birch-pollen extract or a mix of 20-mer peptides as described above. Cells were then harvested and stained with 1 μ g/mL CD123-APC (clone 9F9) and 1 μ g/mL CD63-PE/Cy7 (clone AD1, both from BD Biosciences) for 20 min at 4°C and analysed by flow cytometry as described above.

Measurements of cytokines and tryptase

Culture medium from 6-well plates (2 mL) from the grass extract experiments was concentrated approxi-

mately 10 times by spinning in Amicon filter tubes (Millipore), whereas culture medium from 24-well plates (400 μ L) from the birch extract, and peptide experiments were analysed unconcentrated. Cytokine measurements were performed with Bio-Plex Human Cytokine Assay (Bio-Rad laboratories, Richmond, CA, USA) as described by the manufacturer and analysed with a Luminex instrument (Luminex xMAP Technology, Bio-Rad laboratories). Values below the detection limit on each plate were set to 0. Tryptase measurements were performed using ImmunoCAP Tryptase (Thermo Scientific, Rockford, IL, USA). Tryptase measurements were performed using ImmunoCAP Tryptase (Phadia, Uppsala, Sweden).

Histology and evaluation of eosinophils

Acetone-fixed cryosections (4 μ m) of nasal mucosa were prepared from cultured mucosal tissue, and histological examination of acetone-fixed tissue sections stained with haematoxylin and eosin was performed. Eosinophils were stained with FITC-labelled bovine serum albumin (BSA) (0.8 μ g/mL) as previously described [11].

Statistics

Two-tailed Wilcoxon signed-rank test and Mann-Whitney *U*-test were performed to compare differences within one group or differences between the test groups, respectively, using the Graphpad Prism 5.0 software (Graphpad Software, La Jolla, CA, USA). A *P*-value of < 0.05 was considered significant. To evaluate the relationship between levels of different cytokines, the Spearman rank correlation coefficient and the 95% percentile bootstrapped confidence interval (CI, 1000 resamples), as well as the *P*-value for the Spearman correlation value, were calculated using IBM SPSS (version 22.0) (SPSS Statistics/IBM Corp, Chicago IL, USA).

Results

Increased numbers of Th2-polarized CD4⁺ T cells reside in the steady-state nasal mucosa of allergic subjects

To assess whether Th2-polarized CD4⁺ T cells are constitutively present in the nasal mucosa, surgical specimens from patients with pollen-induced allergic rhinitis and healthy donors were obtained from septum deviation surgery performed outside of the pollen season. The mucosal tissue was digested with Liberase to release cells, and CD4⁺ T cells were readily identifiable by flow cytometry (Fig. 1a). Tissue-derived T cells primarily (90–95%) expressed the memory marker CD45RO (not shown), thus ruling out substantial contamination of

blood T cells that contain a large fraction of naïve T cells. Following stimulation with PMA/ionomycin to induce cytokine synthesis, we detected a discrete population of mucosal CD4⁺ T cells expressing IL-13 that were significantly enriched in allergic individuals compared to healthy controls (Fig. 1b). Furthermore, we consistently observed a small population of IL-5⁺ T cells. This cytokine was mostly co-expressed with IL-13 in the mucosal T cells. Although the fraction of T cells expressing Th2 cytokines tended to be higher in the tissues from allergic individuals, this difference did not reach statistical significance. Analysis of CD8⁺ T cells from these specimens revealed a distinct population of cells expressing IL-13 (Figure S1); however, such cells were not enriched in samples from allergic individuals. IL-5 was only found to be co-expressed with IL-13 in CD8⁺ T cells from one allergic donor.

IL-5 is secreted from allergen-challenged mucosal samples of allergic rhinitis patients ex vivo

To determine whether allergen-reactive T cells resided in the nasal mucosa under steady-state conditions, we

established a method to culture mucosal specimens *ex vivo*. Initial assessments revealed that the morphology of mucosal tissue was well maintained for more than 24 h in culture (Fig. 2). A 24-h culture period was therefore used in all the following experiments. First, we analysed patients with grass-pollen-induced allergic rhinitis ($n = 13$) and non-allergic controls ($n = 8$). Mucosal specimens, obtained out of pollen season, were cultured with or without the presence of grass-pollen extract, and cytokines were measured in the supernatants after 24 h. Analysis revealed that IL-5 levels were significantly increased ($P = 0.03$) in the culture medium of grass-pollen-stimulated samples from allergic patients compared to their non-stimulated counterparts (Fig. 3a). Moreover, no IL-5 was detected in any of the culture supernatants from non-allergic individuals. IL-13 levels were increased in 5 of the patients (Fig. 3b), and there was a positive correlation (r_s 0.84, 95% CI [0.629, 0.967], $P \leq 0.01$) between levels of IL-13 and IL-5 (Fig. 3c). IL-4 (Fig. 3d) and the Th1 cytokine interferon (IFN)- γ (Fig. 3e), as well as TNF- α (data not shown), were detected in most experiments, but there was no significant difference between allergen-stimu-

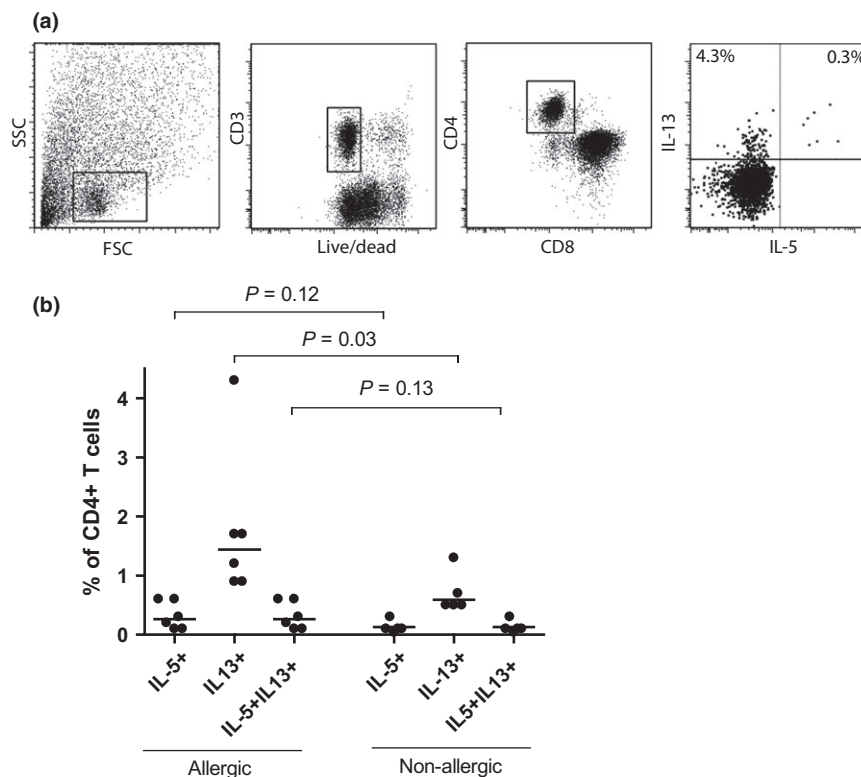


Fig. 1. Detection of Th2-polarized CD4⁺ T cells in steady-state nasal mucosa. Samples of enzymatically digested nasal mucosa contained readily identifiable and viable CD3⁺CD4⁺ T cells secreting IL-5 and IL-13 following PMA/ionomycin stimulation. Gating strategy and dot plots from an allergic donor are shown. Gates defining cytokine production were set based on unstimulated cells (a). Median percentage of nasal mucosal CD4⁺ T cells producing Th2 cytokines compiled from allergic ($n = 6$) and non-allergic ($n = 5$) donors (b). Percentages indicate the fraction of cells producing either IL-5, IL-13 or both cytokines (IL-5⁺ IL-13).

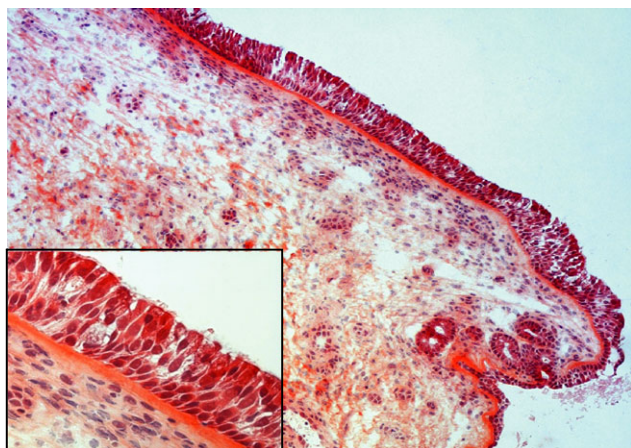


Fig. 2. Haematoxylin-and-eosin-stained section of explant sample cultured for 24 h staining. Note well-preserved respiratory epithelium with cilia (insert).

lated and allergen-unstimulated conditions. The immunoregulatory cytokine IL-10 was also detected in most samples, but the level did not change in response to allergen exposure (Fig. 3f).

Although allergen-reactive T cells were the most likely source of IL-5, we could not exclude the possibility that IL-5 was secreted by other cells, such as mast cells or eosinophils [12–15]. Under homeostatic conditions, the nasal mucosa contains T cells and mast cells, but eosinophils are not regularly observed. Indeed, by *in situ* staining of cryosections from those samples which produced the highest levels of IL-5, only a median of 36 eosinophils per mm² was detected ($n = 5$), whereas T cells were found in high numbers as previously shown¹¹. To determine whether mast cells showed signs of activation, we measured tryptase in the supernatants of samples cultured with and without grass extract from 5 allergic and 4 non-allergic individuals. Tryptase was detected in all samples, but did not increase in response to allergen in either group (Fig. 3g). To directly demonstrate Ag-specific IL-5 production in mucosal T cells, efforts were made to detect cytokines in mucosal biopsies dispersed with several different blends of collagenase (including Liberase) followed by grass-pollen stimulation. However, no apparent cytokine production was detected (data not shown), which was likely due to proteolytic cleavage of molecules on T cells or APCs involved in T-cell activation.

Mucosal samples from birch-allergic patients release IL-5 when challenged with Bet v1-derived peptides

Pollen extract contains a mixture of proteins and other constituents that may stimulate biopsy cells by multiple mechanisms including cross-linking of membrane-bound IgE. To obtain further evidence that IL-5 was

secreted from allergen-reactive T cells, we stimulated nasal biopsies with short peptides that are able to be presented to T cells, but unable to cross-link IgE bound to mast cells. Furthermore, these short peptides do not possess known general immunostimulatory activity, which may be present in allergen extracts [5]. Bet v1 is the major allergen in birch-pollen allergy, and most birch-allergic individuals have circulating Bet v1-reactive T cells [16]. To directly assess whether the synthetic 20-mer peptides spanning Bet v1 could potentially activate mast cells, we purified basophils from peripheral blood of birch-allergic individuals, treated the cells with birch allergen extract or a mix of 20-mer peptides and assessed the subsequent membrane translocation of CD63. Indeed, we observed CD63 translocation only in response to the extract (Fig. 4a), thus clearly indicating that the peptides lack mast-cell activation capacity. We observed no CD63 staining on treated basophils from healthy controls (not shown). Further, we obtained mucosal tissue from patients with well characterized birch-pollen-induced allergic rhinitis ($n = 11$) and non-allergic controls ($n = 8$) and cultured parallel samples challenged with birch-pollen extract or a Bet v1-derived peptide mix compared with unchallenged control samples. Analysis revealed that IL-5 was secreted in the supernatants from 45% of the samples from birch-allergic patients in response to birch extract (Fig. 4b), similar to that observed with grass extracts. Importantly, we found that specimens from four of five allergic individuals that secreted IL-5 in response to birch-pollen extract also secreted IL-5 in response to the Bet v1 peptide mix (Fig. 4c). In addition, one individual produced IL-5 only in response to peptides. Moreover, in 4 of 5 supernatants with peptide-induced IL-5 production, a concurrent increase in IL-13 secretion was observed (Fig. 4d). IL-10 and TNF- α were detected in most samples, but there were no difference between unchallenged and challenged conditions (not shown). IL-4 and IFN- γ levels were below the detection threshold (not shown). The cytokine level in supernatants following birch challenge was generally lower than in experiments using grass extract and was likely due to the fact that cytokines in the former experiments were measured in unconcentrated supernatants from smaller biopsy samples.

Discussion

Here, we show that allergen-specific Th2 cells are residing in the nasal mucosa of allergic rhinitis patients out of the allergy season and that such cells secrete Th2 cytokines upon allergen exposure. Together, our findings indicate that local activation of allergen-specific Th2 cells, normally residing in the nasal mucosa, is an

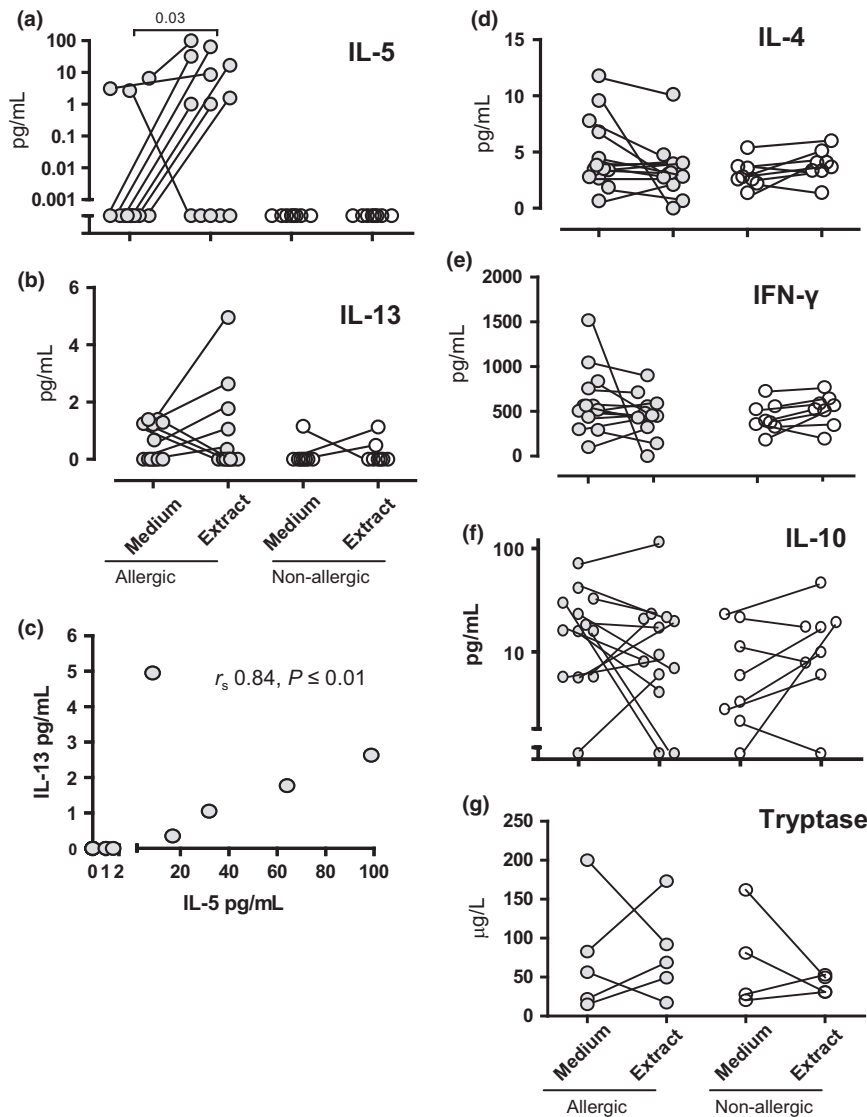


Fig. 3. Cytokine secretion after 24 h culture with or without grass-pollen allergen. IL-5 (a), IL-13 (b), IL-4 (d), IFN- γ (e) and tryptase (f) were measured in the supernatants of tissue samples from allergic rhinitis subjects ($n = 13$), termed allergic (grey symbols) and healthy control subjects ($n = 8$), termed non-allergic (white symbols) cultured with grass-pollen extract or medium control samples. (c) Correlation between IL-5 and IL-13 in supernatants of challenged samples from allergic patients. Note that the 6 subjects lacking cytokine response to neither cytokine are clustered in (0,0).

early component of the inflammatory pathway in seasonal upper airway allergy.

Activated allergen-specific Th2 cells are believed to play a central role in the inflammatory reaction, both in allergic rhinitis and allergic asthma [2, 3, 5]. Studies of experimentally induced rhinitis have shown that the mRNA expression of several Th2-associated cytokines increased in mucosal CD4⁺ T cells 24 h after challenge [7, 14, 17]. However, these *in vivo* models did not address the question of where and how these T cells become activated. It is well documented that airway DCs capture aeroallergens locally and subsequently migrate to the draining lymph nodes and stimulate

allergen-specific T cells [17]. This DC-T-cell interaction imprints trafficking molecules on the T cells, instructing them to preferentially 'home' back to the site of challenge [18]. Alternatively, memory T cells residing in the effector tissues, including tissue-resident memory T cells (Trm), may be activated locally [19]. These are important issues to address in order to therapeutically target early events in the inflammatory pathway. We found that outside of the allergy season, the nasal mucosa of allergic rhinitis patients contained a significantly higher percentage of Th2 cells than healthy controls. Moreover, the majority of such Th2 cells produced IL-13, whereas IL-5⁺ T cells constituted a

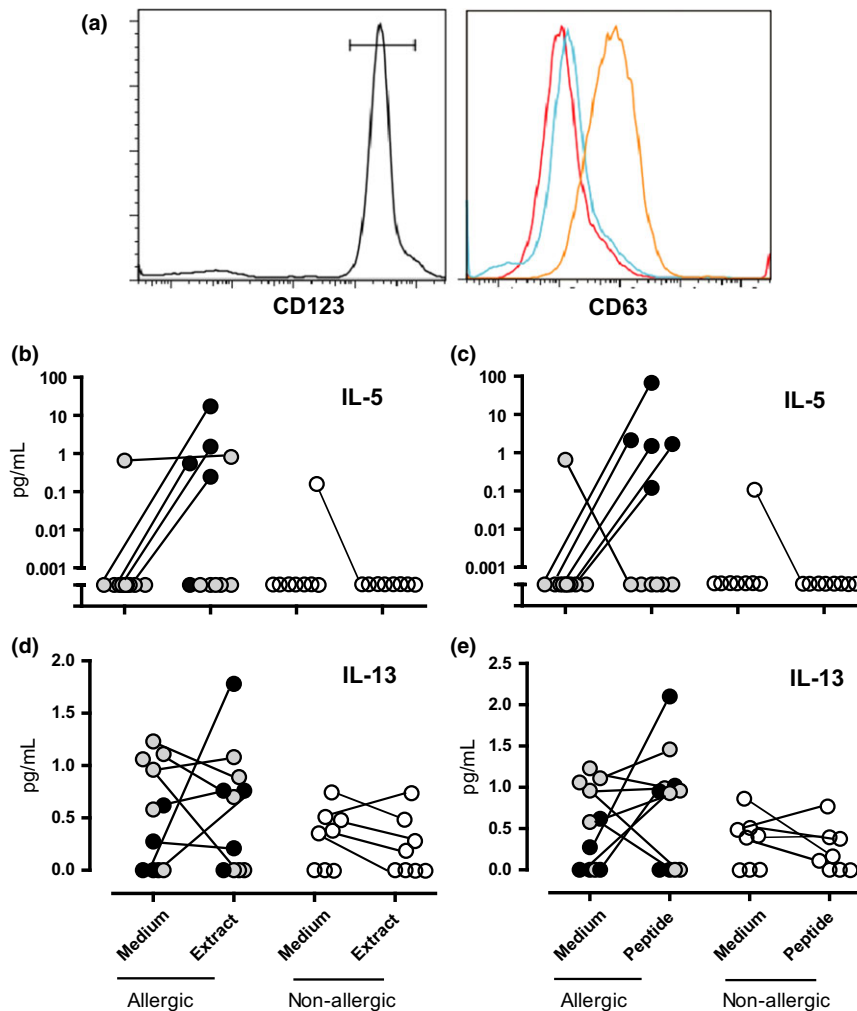


Fig. 4. Cytokine secretion after 24-h culture with or without birch-pollen allergen or Bet v1-derived peptides. (a) Enriched basophils (CD123+, left panel) from PBMCs of birch-allergic donors were treated with birch allergen extract or 20-mer peptides spanning Bet v1, and analysed by flow cytometry for CD63 surface membrane translocation (right panel). Blue histogram, untreated cells; red histogram, peptide-treated cells; orange histogram, extract-treated cells. Results from one of two birch-allergic donors shown. IL-5 (b, c) and IL-13 (d, e) were measured in the supernatants of tissue samples from allergic rhinitis subjects ($n = 11$), termed allergic (grey or black symbols) or healthy control subjects ($n = 8$), termed non-allergic (white symbols) cultured with grass-pollen extract or medium control samples. The cultures that secreted IL-5 in response to Bet v1-derived peptides are indicated (black symbols).

minor population, and IL-5 production was mostly observed in cells co-expressing IL-13. This is compatible with the reported heterogeneity of Th2 cells [20], in which IL-5 production appears confined to a minor population of Th2 cells co-expressing IL-13 and IL-4.

Using a tissue explant model, we were able to selectively study how resident cells responded to allergen exposure without contribution from the systemic compartment. This model has previously been used by others, and in agreement with our results, increased mRNA levels for Th2 cytokines have been detected in T cells in response to allergen extract exposure [21]. However, in the previous report, T cells accounted for only 44% of IL-5 mRNA⁺ cells in response to ragweed, suggesting that other cells were important producers of IL-5. Both

mast cells and eosinophils express the high-affinity receptor for IgE which may be cross-linked by pollen extracts. Activation of these cell types (and other cells) may therefore also lead to production of IL-5 and/or contribute to the activation of local T cells. To selectively study antigen-dependent activation of resident allergen-reactive T cells, we therefore exposed the explanted mucosa to a 20-mer overlapping peptide mixture spanning the Bet v1 protein. Bet v1 is a major T-cell epitope for birch-allergic individuals, and we found that these short peptides do not cross-link membrane-bound IgE, thus excluding the possibility of IgE-dependent mast-cell or eosinophil activation. Using this approach, we were able to show that allergen-specific T cells secreted significant amounts of IL-5, and some

also IL-13, that were detected in the culture medium. Moreover, in parallel experiments, we found almost completely overlap of increased levels of IL-5 in response to extract and peptides, strongly suggesting that T cells were the major source of Th2 cytokines in both situations.

It is well accepted that an allergic inflammatory reaction leads to increased recruitment of immune cells from the circulation. Moreover, we recently reported that the steady-state trafficking of T cells to the nasal mucosa depends on the chemokine CCL28 [22], whereas other studies have found that T-cell recruitment during allergic inflammation rather depends on the CCR4-binding chemokines CCL17 and CCL20 [18]. It is therefore surprising that *in vivo* models of allergic rhinitis show increased numbers of activated T cells in the nasal mucosa, but virtually no or only marginal increase in total T-cell numbers [4, 23]. This may either imply that the transit time for T cells in the mucosal tissue is accelerated in response to allergen exposure, or alternatively, that the migration of T cells into the tissue is very restricted, and that allergen exposure rather activates Th2 cells locally. The latter notion is compatible with our findings and is in line with the emerging concept of Trms as a major component of memory lymphocyte populations in diverse peripheral tissue sites, including mucosal tissues [19]. In fact, we find that the majority of nasal mucosal T cells express CD69 (Bækkevold, unpublished), which is a functional marker of tissue T-cell retention. To this end, it will be important to define the factors regulating specific homing and tissue retention of Th2 cells, including the CCR8-ligand CCL8, which has been implicated in selective recruitment of IL-5⁺ T cells [24]. Moreover, this suggests that local activation of resident T cells or inflammatory pathways upstream of such activation should be targets for therapeutic intervention.

In our experiments, explant samples from approximately 50% of patients with allergic rhinitis secreted significant amounts of IL-5, both with grass and birch

extract as well as with Bet v1-derived peptides. This is likely a result of the limited size of the tissue samples that can be obtained from the mucosa, and it is reasonable to assume that although the mucosa contains allergen-specific Th2 cells, the secretion of cytokines in response to allergen exposure was under the threshold for detection.

We could readily detect secretion of a number of cytokines not associated with Th2-responses in the explant supernatants, including TNF- α and IL-10, which were not regulated in response to allergen exposure. This is likely related to the tissue damage induced during biopsy, which induces high production of proinflammatory cytokines and factors involved in tissue healing regeneration.

In conclusion, our *ex vivo* model shows that significant amounts of the Th2-associated cytokine IL-5 (and IL-13) were secreted from resident cells in response to allergen challenge out of season. Production of IL-5 and IL-13 in response to Bet v1-derived peptides strongly suggested that T cells are the main source of these Th2 cytokines. IL-5 is an important cytokine for recruitment and activation of eosinophils, and signalling pathways upstream of IL-5 secretion could therefore be an important target for anti-allergic therapy.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical characterization of allergic subjects.

Figure S1. Detection of Th2-polarized CD8⁺ T cells in steady-state nasal mucosa.