

Cytokine expression during allergen-induced late nasal responses: IL-4 and IL-5 mRNA is expressed early (at 6 h) predominantly by eosinophils

K. T. NOURI-ARIA, F. O'BRIEN, W. NOBLE, M. R. JACOBSON,
K. RAJAKULASINGAM* and S. R. DURHAM

Upper Respiratory Medicine, National Heart & Lung Institute, and *Homerton Hospital, London, UK

Summary

Background The production of TH₂-type cytokines [interleukin-4 (IL-4) and IL-5] and tissue eosinophilia are characteristic features of allergic diseases. It was previously reported that at 24 h after allergen provocation, CD3⁺ T-lymphocytes were the principal cell source of IL-4 and IL-5 mRNA transcripts in both atopic asthma and rhinitis.

Objectives To investigate whether IL-4 and IL-5 mRNA are expressed earlier during late nasal responses and if so, which cell(s) are responsible.

Methods Nasal biopsies were obtained at 6 h after nasal allergen challenge and following a control challenge with the allergen diluent. Sections were immunostained for T-lymphocytes (CD3⁺, CD4⁺) and eosinophils (EG2⁺). *In situ* hybridization was used to detect the number of cells expressing messenger RNA (mRNA) for IL-4 and IL-5.

Results In patients with allergic rhinitis, eosinophils (EG2⁺ cells $P = 0.006$) but not T-cells (CD3⁺ cells) increased in the nasal mucosa at 6 h after allergen challenge. The number of cells expressing IL-4 mRNA ($P = 0.01$) and IL-5 mRNA ($P = 0.05$) also increased at 6 h. Co-localization studies showed that 76% of IL-4 mRNA⁺ cells and 77% of IL-5 mRNA⁺ cells were eosinophils, whereas at this time point, T-cells and mast cells accounted for $\leq 5\%$ of mRNA expression; the identity of the remaining 20% of IL-4 and IL-5 mRNA⁺ cells was not determined. By use of immunohistology, cytokine protein expression at 6 h was confirmed for IL-4 but not for IL-5. No increases in T-cells, eosinophils or cytokine expression were detected in non-atopic subjects.

Conclusions Eosinophils represent an early source of IL-4 which may contribute to TH₂-type responses during late nasal responses and ongoing allergic rhinitis.

Keywords: allergic rhinitis, TH₂ cytokines, eosinophils, allergen challenge, immunohistochemistry, *in situ* hybridization

Clinical and Experimental Allergy, Vol. 30, pp. 1709–1716. Submitted 28 July 1999; revised 8 November 1999; accepted 24 February 2000.

Introduction

Characteristic features of allergic rhinitis include the IgE-dependent release of mediators from mast cells and infiltration of the nasal mucosa with eosinophils [1,2]. These events appear to be controlled, at least in part, by cytokines such as IL-3, IL-4 and IL-5, which are produced

by activated T-lymphocytes, mast cells and by eosinophils [3,4]. These cytokines are critical for eosinophil recruitment and IgE expression [5,6,7], and represent potential targets for therapeutic intervention in allergic disorders. The production of these cytokines following allergen exposure is likely to vary according to the phenotype and time course of infiltrating cells. Cell kinetics have been studied in the skin during late responses, where it is feasible to examine multiple biopsies obtained following allergen provocation. Results indicated that whereas cutaneous

Correspondence: S. R. Durham, Upper Respiratory Medicine, Imperial College School of Medicine at The National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, UK.

eosinophils peaked at 6 h, T-lymphocytes were maximal at 6–24 h and macrophages at 48 h [8,9]. The production of cytokines paralleled the clinical late response and were maximal at 6 h after challenge. It has previously been confirmed that in the target organ in patients with allergic rhinitis, IL-4 and IL-5 were similarly expressed in the nasal mucosa and at 24 h, the only time point examined, T-lymphocytes were the principal cell source, with a lesser contribution from mast cells and eosinophils [10,11]. In this study, an earlier time-point, 6 h, after nasal allergen and following a control challenge with allergen diluent was examined. The effect of allergen in healthy non-atopic control subjects was also examined. The basic hypothesis to be tested was that in parallel with the skin, TH₂-type cytokines are expressed early (at 6 h) after allergen and may therefore contribute to the development of late responses. It was also questioned whether alternative celltypes, particularly mast cells [12] and eosinophils [13] rather than T-lymphocytes [14], may represent an earlier cell source of these cytokines.

Materials and methods

Patients

Seventeen patients with seasonal allergic rhinitis due to grass pollen were recruited from the Allergy Clinic and the Nose Clinic at the Royal Brompton Hospital. All patients were non-smokers and had a history of seasonal rhinitis in June through July for at least 2 years and a positive skin-prick test (>5 mm) to Timothy grass pollen extract (Soluprick, *Phleum pratense*, ALK, Horsholm, Denmark). Exclusion criteria included a history of perennial allergies, other significant clinical illness and treatment with immunotherapy in the previous 5 years. Six normal non-atopic subjects (skin test negative to 11 common aeroallergens which included pollens, moulds, animal dander and house dust mite, Soluprick, ALK) were also studied. They were matched for age and gender. Total IgE concentrations and allergen-specific IgE to Timothy grass pollen are included in Table 1.

Study design

Approval was obtained from the hospital ethics committee of the Royal Brompton and Harefield NHS Trust. All patients gave informed written consent. At a preliminary visit, a clinical history was taken, skin prick tests were performed, and blood was taken for total and allergen-specific IgE concentrations. Two biopsies were obtained from each patient (one on the control day 6 h after allergen diluent and the second 6 h after allergen challenge from the opposite nostril). All provocation tests were performed

Table 1. Demographic details of patients studied

	Patients	Normal subjects
Age	27 (24–28)	23 (22–30)
M:F	14:3	5:1
Total IgE* (IU/mL)	267.5 (182–608)	33.5 (17–73)
Allergen Specific IgE* (IU/mL)	20.5 (12–44)	<0.35

*Median (interquartile range).

outside the pollen season and the interval between challenges was a minimum of 3 weeks. In three patients, biopsies were obtained 6 h after allergen and not following a diluent challenge.

Nasal provocation

Nasal challenge was undertaken using a hand-held pump spray which delivered 0.13 mL per actuation to each nostril. The pump was placed in one nostril while occluding the contralateral nostril and activated once during quiet inspiration. The procedure was then repeated in the opposite nostril. A total dose of 1000 biological units (500 BU per actuation) of Timothy grass pollen extract (ALK) was delivered to each nostril. On the control day, the allergen diluent alone was used. Nasal biopsies (2.5 mm) were taken using Gerritsma forceps following local anaesthesia with cocaine 3% and adrenaline 0.025% solution [15]. Patients were observed for 1 h and discharged.

Tissue processing

Nasal biopsies were fixed immediately in freshly prepared 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) for 2 h, followed by 2 × 15% sucrose in PBS for 1 h and overnight, respectively. Biopsies were embedded in OCT (BDH, Poole, UK) and stored at – 80 °C.

Immunohistology

Sections (6 µm) were cut and placed on poly L-lysine-coated slides prior to immunostaining using alkaline phosphatase anti-alkaline phosphatase (APAAP) as previously described [16]. The monoclonal antibodies used were anti-CD3 (total T-cells), EG2 (activated eosinophils) and tryptase AA1 (mast cells). For negative controls,

primary antibodies were replaced with either non-specific mouse IgG or tris-buffered saline.

IL-4 and IL-5 protein

Immuno-reactive IL-4 and IL-5 were detected using biotinylated rat anti-human IL-4 and anti-human IL-5 monoclonal antibodies (Pharmingen, BD, Oxford, UK). Sections were permeabilized by washing in freshly prepared 0.1% saponin in PBS for 30 min. Sections were then treated with avidin/biotin blocking kit (Vector Laboratories Ltd, Peterborough, UK) to block non-specific binding of biotin/avidin system reagents. Slides were incubated with avidin D blocking solution for 15 min, rinsed briefly in saponin/PBS and then incubated for 15 min with the biotin blocking solution. The sections were incubated overnight at room temperature with rat anti-human IL-4 or IL-5 monoclonal antibodies or isotype-matched monoclonal antibody of irrelevant specificity (Pharmingen) diluted in saponin/PBS containing 150 μ L rabbit serum per 10 mL PBS. Slides were then washed with saponin/PBS and incubated for 30 min with biotinylated rabbit anti-rat IgG (Vector Laboratories) in saponin/PBS/rabbit serum. Sections were then washed with saponin/PBS and incubated for 30 min with Vectastain ABC-AP (Vector Laboratories). Sections were then washed and incubated for 20 min with fast blue BB salt or fast red TR salt (Sigma Aldrich Company Ltd, Poole, UK). Slides were then washed in water and mounted in Glycergel (Dako Ltd, Cambridge, UK).

In situ hybridization

Riboprobes, antisense and sense, were prepared from cDNA for IL-4 and IL-5. cDNA was inserted into different pGEM vectors and linearized with appropriate enzymes before transcription. Transcription was performed in the presence of ^{35} S uridine triphosphate (^{35} S-UTP) and the appropriate T7 or SP6 RNA polymerases. For *in situ* hybridization, 6 μ m cryostat sections were processed on poly L-lysine-coated slides. Sections were permeabilized with Triton X-100 in PBS followed by proteinase K digestion. In order to inhibit non-specific binding of ^{35} S, sections were treated with iodoacetamide and N-ethylmaleimide, and then acetic anhydride/triethanolamine [17]. As a negative control, sections were hybridized with the sense probe or treated with ribonuclease-A solution before the prehybridization step with antisense probes. Double immunohistochemistry/*in situ* hybridization was performed on selected biopsies after challenge where adequate numbers of IL-4 or IL-5 mRNA expressing cells were present and adequate number of sections were available for co-localization studies. Sections were first immunostained

for phenotypic markers and then processed for *in situ* hybridization.

Counting method

Slides were counted blind in coded random order using an Olympus BH2 microscope (Olympus Optical Company Ltd, Tokyo, Japan) with an eye piece graticule (0.202 mm² at 200 $\times$ magnification). At least two sections and a minimum of six fields were counted for each monoclonal antibody. The graticule was orientated along the whole length of the epithelial basement membrane extending to a depth of 0.5 mm, which includes the majority of submucosal cells while excluding the majority of submucosal glands. Subepithelial counts were expressed as mean counts per mm². The mean intraobserver coefficient of variation for repeat counts was 8%.

Statistical analysis

Within-group comparisons were performed using the Wilcoxon matched-pairs signed-ranks test. *P*-values <0.05 were considered statistically significant. Statistical tests were performed using a standard computer package (Minitab Inc. State College, PA, USA).

Results

Immunocytochemistry

Cell infiltration

In patients with allergic rhinitis, the median number [interquartile ranges (IQ)] of EG2⁺ cells in the nasal submucosa on the control day was 7 (1, 13) cells/mm² and was similar to the corresponding values on the saline challenge day in non-atopic normal subjects 5 (0, 14) cells/mm². When compared with baseline on the allergen challenge day (Fig. 1), there was a significant increase in the number of EG2⁺ cells, 34 (12.4, 88.5) (*P* = 0.006). When these changes (allergen–diluent) were compared between patients and normal subjects, significant increases were seen for EG2⁺ cells (*P* = 0.009). In contrast, no increases in EG2⁺ cells were observed in the normal subjects 8 (4, 12) (*P* = 1.0). For both groups, the numbers of CD3⁺ T-cells and tryptase⁺ mast cells in the nasal mucosa were comparable after the control challenge and did not change significantly after allergen provocation (Fig. 1).

Cytokine expression

It was determined whether allergen-induced increases in cytokine mRNA expression for IL-4 and IL-5 were

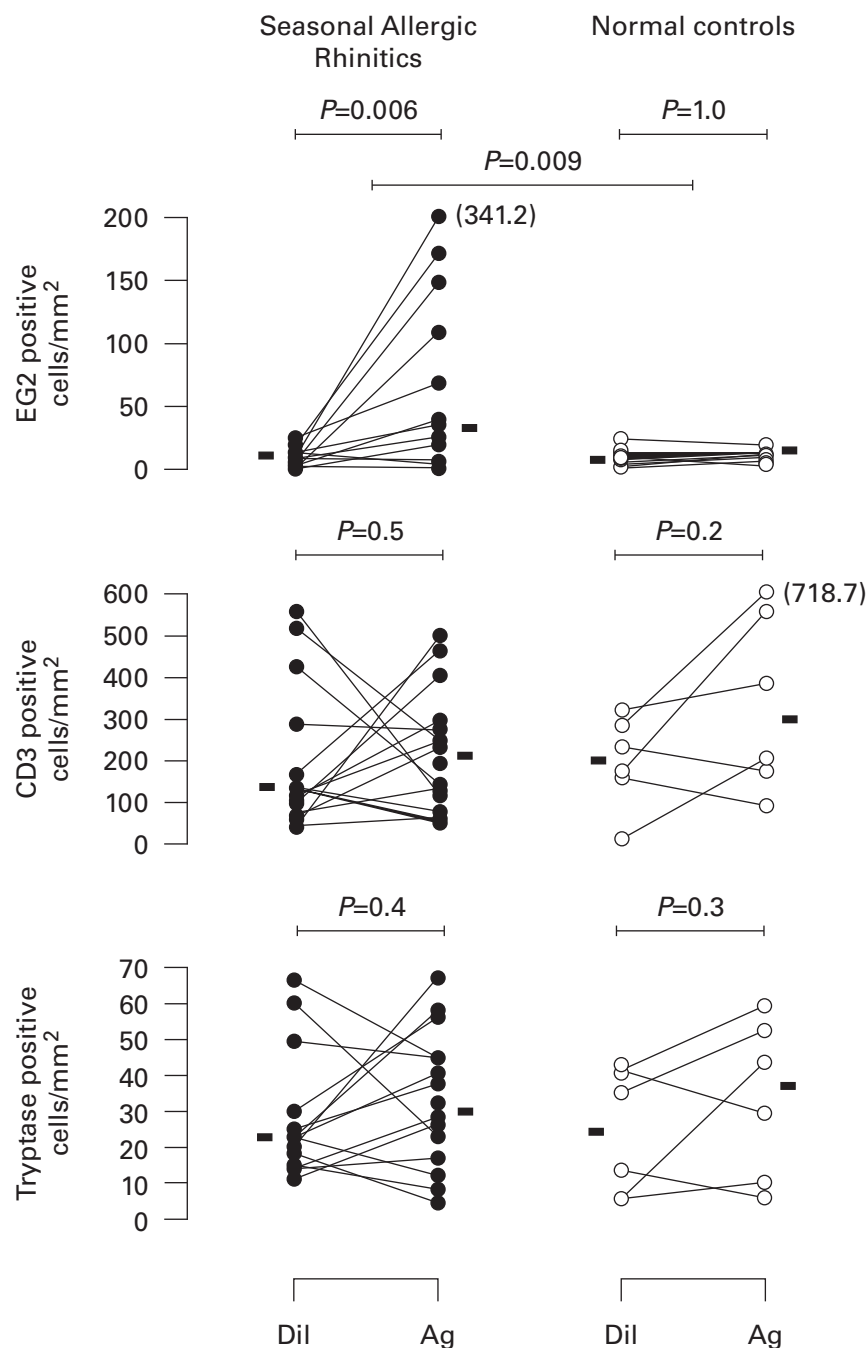


Fig. 1. Cell counts for activated eosinophils (EG2), T-cells (CD3) and mast cells (tryptase) in the nasal mucosa 6 h after allergen challenge in seasonal allergic rhinitis and normal controls for diluent (Dil) and allergen (Ag) challenge. Levels of statistical significance are shown.

accompanied by expression of cytokine product. Cells expressing IL-4 and IL-5 protein were assessed by use of immunohistochemistry in biopsies, where substantial numbers of cytokine mRNA expressing cells had been observed. In five biopsies studied, the median/mm² (interquartile range) IL-4 positive cells were 28 (2.5, 200). Although double immunohistochemistry to identify the cell source of immune-reactive IL-4 protein was not performed owing to lack of adequate sections, the majority

of IL-4 protein positive cells exhibited the characteristic morphology of eosinophils (Fig. 4b). No IL-5 protein was detected at 6 h in any of the five biopsies tested.

In situ hybridization

Nasal biopsies were hybridized with sense and antisense riboprobes encoding for IL-4 and IL-5. Cells expressing IL-4 and IL-5 mRNA were identified as discrete clusters of

silver grains distributed evenly throughout the lamina propria and deeper in the submucosal layers. The specificity of the signals was confirmed by the absence of hybridization signals with corresponding sense probes (with identical sequence to mRNA), or pre-treatment of sections with RNase prior to hybridization.

IL-4 mRNA⁺ cells were seen only occasionally after diluent challenge for both allergic [median 0 (IQ 0–3)] and non-allergic subjects [0 (0–5)]. In patients with allergic rhinitis, there were significant increases after allergen in the number of cells expressing IL-4 mRNA [13 (0–40)] ($P = 0.01$), whereas in normal subjects [1.65 (0–15)] no change in IL-4 mRNA expression was observed ($P = 0.4$) (Fig. 2).

A similar pattern was seen for IL-5 mRNA expression, with significant increases after allergen challenge for the allergic group ($P = 0.05$) but no change observed in normal controls ($P = 0.5$) (Fig. 2). Between-group comparisons for these increases in IL-4 and IL-5 failed to achieve statistical significance ($P = 0.2$ and $P = 0.1$, respectively), possibly reflecting the relatively small number of control subjects studied.

Co-localization of IL-4 mRNA⁺ and IL-5 mRNA⁺ cells

In order to determine the phenotype of IL-4 and IL-5 mRNA⁺ expressing cells, sequential immunohistochemistry and *in situ* hybridization were performed on five biopsies obtained 6 h after allergen challenge. A large proportion of IL-4 mRNA⁺ cells were co-localized to EG2⁺ cells (76%), a small contribution from CD3⁺ cells (5%) and none from tryptase⁺ cells. Similarly, the majority of IL-5 mRNA⁺ cells were co-localized to EG2⁺ cells (77%) compared with CD3⁺ cells (3.5%) and mast cells (2%) (Fig. 3). The cell sources of the remaining 19% of IL-4 mRNA⁺ cells and 17% of IL-5 mRNA⁺ cells were not identified by this panel of markers. The relative proportions of total eosinophils, T-cells and mast cells that expressed these cytokines were also determined. Over half the eosinophil (EG2⁺) population expressed IL-4 and IL-5, whereas only 1% of T-cells expressed both cytokines, and 1.5% of mast cells expressed IL-5 mRNA (Table 2 and Fig. 4a).

Discussion

Allergen provocation in patients with seasonal allergic rhinitis resulted in an increase at 6 h in the number of eosinophils in the nasal mucosa, whereas CD3⁺ T-lymphocytes and tryptase⁺ mast cells remained unchanged. Allergen-induced increases in cells expressing mRNA for interleukin-4 and interleukin-5 were also detectable at this

time point, when eosinophils represent by far the most dominant cell source of these cytokines, with only a small contribution from T-cells and mast cells. Eosinophils are recruited early after allergen challenge and may contribute to TH₂-type responses and on-going tissue eosinophilia during late nasal responses.

Simultaneous immunocytochemistry/*in situ* hybridization was performed on the same sections in order to identify the phenotype of cells expressing cytokine mRNA [11]. The 6 h time point was chosen on the basis of a previous study, which showed that grass pollen allergen provocation resulted in late symptoms and nasal blockage in 50% of patients between 3 and 10 h [18]. Also, previous studies in the skin [19] and nose [20] have shown a peak of eosinophils at 6 h after challenge. The present findings at 6 h are in contrast to previous findings in the nasal mucosa at 24 h, where the principal cell sources of IL-4 and IL-5 were CD3⁺ T-cells (approximately 70%) with lesser contributions from mast cells (15–20%) and eosinophils (0–5% IL-5 and no IL-4 expression) [10,14]. Thus, at 6 h as opposed to 24 h, eosinophils may be the dominant cytokine-producing cells within nasal mucosa.

Immunohistochemistry using monoclonal antibodies specific for cytokines confirmed that the increase in IL-4 mRNA detected at 6 h after allergen challenge was accompanied by expression of IL-4 protein, whereas this was not the case for IL-5. One possible explanation may be a shorter time course for mRNA translation for IL-4 compared with IL-5. For example, detection of IL-4 protein in supernatant fluids of fully differentiated human T-cell clones peaks at 4–6 h, whereas IL-5 protein peaks at 18–24 h (A. Verhoef, personal communication).

Previous studies of the kinetics of allergen-induced cutaneous responses have shown that the size of the late response peaks at 6–24 h after challenge [8,21,22]. A time-dependent increase in both inflammatory cells [21] and cytokine mRNA positive cells [8] was observed at 6–24 h, although in the latter study the cell source of cytokines was not examined. In a further study of late cutaneous biopsies [19], the principle cell sources of both IL-4 and IL-5 mRNA were T-cells, with a lesser contribution from eosinophils and mast cells. However, the respective contribution of these cells to cytokine expression at 6 h compared with 24 h was not determined. Nevertheless, these studies in the skin confirm the early infiltration of eosinophils, and raise the possibility that eosinophils may represent an alternative earlier source of both IL-4 and IL-5 during human late allergic responses. The role of basophils as an alternative initial cell source of IL-4 [23] during the late nasal response cannot be excluded, particularly since the phenotype of approximately 20% of IL-4 and IL-5 mRNA producing cells in the present nasal

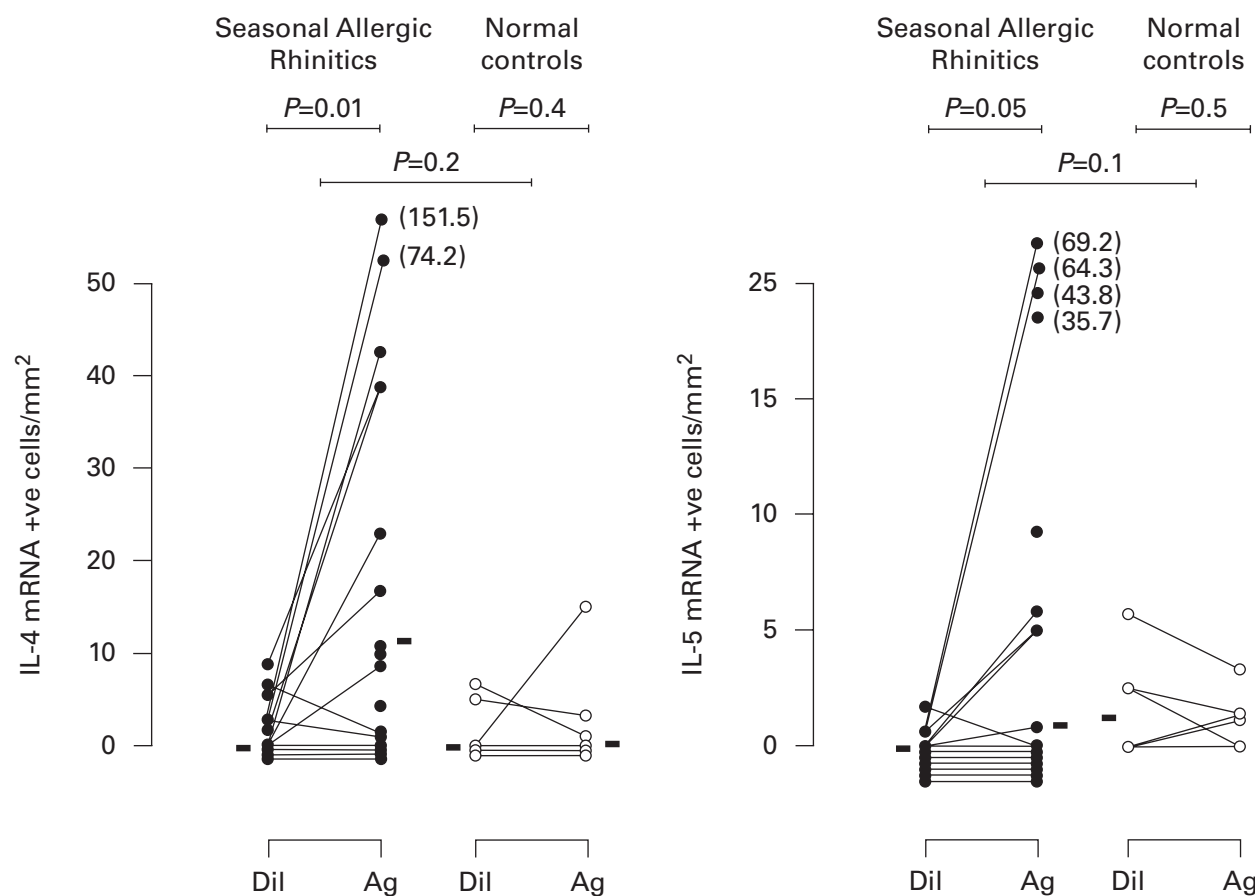


Fig. 2. IL-4 and IL-5 mRNA expression at 6 h after allergen challenge in seasonal allergic rhinitis and normal controls for diluent (Dil) and allergen (Ag) challenge. Levels of statistical significance are shown.

biopsies were not co-localized with the monoclonal antibodies used.

Eosinophils, prominent cells in asthmatic inflammation and allergic diseases, have been reported to synthesize, store and release many cytokines and growth factors, including IL-4, IL-5, IL-6, IL-2, IFN γ and IL-12 [24–28]. This diversity in cytokine secretion by eosinophils illustrates their multifunctional potential in allergic inflammation. The early recruitment of eosinophils following immediate allergen-induced responses may occur as a consequence of release of chemotactic factors following IgE-dependent mast cell activation (e.g. histamine, PAF, LTB₄) [29]. Also, mast cell-derived IL-4 might selectively upregulate VCAM-1 expression [30], inducing a preferential eosinophil adhesion to vascular endothelium. It has been shown that eosinophils represent an early autocrine source of IL-5, with potential to initiate/amplify the late phase response and, via IL-4, to preferentially recruit/activate TH₂-type CD4⁺ lymphocytes which characterize late responses [19]. Autocrine production of IL-5 by eosinophils may also contribute to prolonged eosinophil survival by

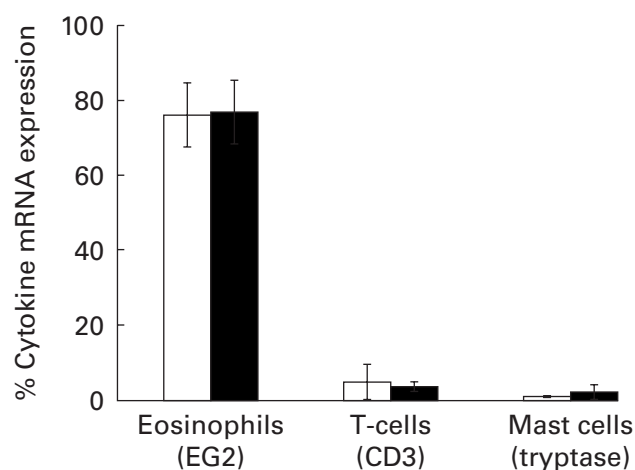


Fig. 3. Phenotype of cells expressing cytokines mRNA. Percentages of IL-4 (□) and IL-5 (■) mRNA⁺ cells (mean $\pm$ SD) which co-express phenotypic markers for T-lymphocytes (CD3), mast cells (tryptase) and activated eosinophils (EG2).

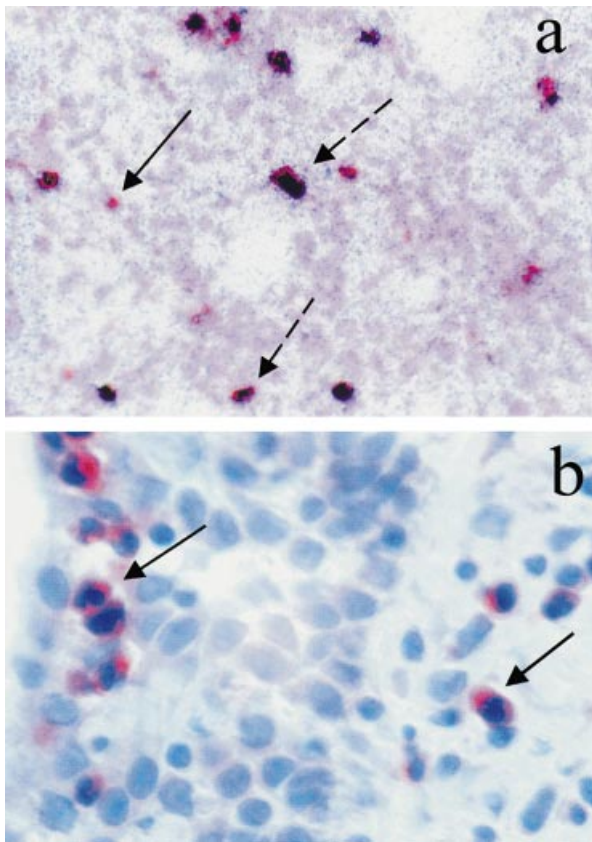


Fig. 4. (a) Co-localization of IL-4 mRNA to eosinophils using *in situ* hybridization/immunohistochemistry. Broken arrows show EG2⁺ eosinophils expressing IL-4 mRNA. The solid arrow shows an EG2⁺ eosinophil without IL-4 mRNA expression. (b) Immunostaining for IL-4 protein in a nasal biopsy obtained 6 h after allergen challenge. Solid arrows show IL-4 positive cells.

inhibition of eosinophil apoptosis [31]. Eosinophils produce several potent proteins, including major basic protein (MBP) and eosinophil cationic protein (ECP) which are known to be toxic to the human respiratory epithelium [32]. Eosinophils represent an abundant source of the sulphido-peptide leukoterines LTC₄, LTD₄ and LTE₄ which are known to increase mucus production and cause airway narrowing [33].

Table 2. Median percentage EG2⁺, Tryptase AA1⁺ and CD3⁺ cells co-expressing IL-4 mRNA and IL-5 mRNA

	% EG2 ⁺ cells	% Tryptase ⁺ cells	% CD3 ⁺ cells
IL-4 mRNA	70*	0	0
(IQ range)	(48–81)	(0–0)	(0–3.37)
IL-5 mRNA	51.4	1.5	1.5
(IQ range)	(48–55)	(0–3.75)	(0.2–3)

*Median (interquartile range).

The early recruitment of eosinophils, and their potential in providing an earlier source of IL-4 and IL-5 during late responses, highlights this cell type as an important therapeutic target in the treatment of allergic diseases.

Acknowledgements

This work was supported by National Asthma Campaign (UK).

References

- 1 Naclerio RM. Allergic rhinitis. *N Engl J Med* 1991; 325:860–9.
- 2 Bentley AM, Jacobson MR, Cumberworth V *et al*. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992; 89:877–83.
- 3 Bradding P, Roberts JA, Britten KM *et al*. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10:471–80.
- 4 Moqbel R, Ying S, Barkans J *et al*. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol* 1995; 155:4939–47.
- 5 Moqbel R, Hamid QA, Ying S *et al*. Expression of mRNA and immunoreactivity for granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J Exp Med* 1991; 174:749–52.
- 6 Durham SR, Gould HJ, Thienes CP *et al*. Expression of epsilon germ-line gene transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur J Immunol* 1997; 27:2899–2906.
- 7 Del Prete GE, Maggi P, Parronchi I *et al*. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988; 140:4193–8.
- 8 Tsicopoulos A, Hamid Q, Haczku A *et al*. Kinetics of cell infiltration and cytokine messenger RNA expression after intradermal challenge with allergen and tuberculin in the same atopic individuals. *J Allergy Clin Immunol* 1994; 94:764–72.
- 9 Ying S, Barata LT, Meng Q *et al*. High affinity immunoglobulin E receptor (Fc ϵ RI) bearing eosinophils, mast cells, macrophages and langerhan's cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *Immunology* 1998; 93:281–8.
- 10 Ying SSR, Durham MR, Jacobson S *et al*. T lymphocytes and mast cells express messenger RNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994; 82:200–6.
- 11 Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB. Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. *Am J Respir Cell Mol Biol* 1995; 12:477–87.

- 12 Bradding P, Feather IH, Wilson S *et al.* Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993; 151:3853–65.
- 13 Moqbel R, Levi SF, Kay AB. Cytokine generation by eosinophils. *J Allergy Clin Immunol* 1994; 94:1183–8.
- 14 Ying SSR, Durham J, Barkans K *et al.* T cells are the principal source of interleukin-5 mRNA in allergen-induced rhinitis. *Am J Respir Cell Mol Biol* 1993; 9:356–60.
- 15 Fokkens WJ, Vroom TM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. *Rhinology* 1998; 26:293–5.
- 16 Varney VA, Hamid QA, Gaga M *et al.* Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993; 92:644–51.
- 17 Kay AB, Ying S, Varney V *et al.* Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991; 173:775–8.
- 18 Varney VA, Jacobson MR, Sudderick RM *et al.* Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992; 146:170–6.
- 19 Barata LT, Ying S, Meng Q *et al.* IL-4- and IL-5-positive T lymphocytes, eosinophils, and mast cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Allergy Clin Immunol* 1998; 101:222–30.
- 20 Rajakulasingam K, Durham SR, O'Brien F *et al.* Enhanced expression of high-affinity IgE receptor (Fc epsilon RI) alpha chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. *J Allergy Clin Immunol* 1997; 100:78–86.
- 21 Frew AJ, Kay AB. The relationship between infiltrating CD4⁺ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J Immunol* 1988; 141:4158–64.
- 22 Frew AJ, Kay A. B. The pattern of human late-phase skin reactions to extracts of aeroallergens. *J Allergy Clin Immunol* 1998; 81:1117–1121.
- 23 Irani AA, Huang C, Xia H-Z *et al.* Immunohistochemical detection of human basophils in late-phase skin reactions. *J Allergy Clin Immunol* 1998; 101:354–8.
- 24 Lacy P, Levi-Schaffer F, Mahmudi-Azer S *et al.* Intracellular localization of interleukin-6 in eosinophils from atopic asthmatics and effects of interferon gamma. *Blood* 1998; 91:2508–16.
- 25 Levi-Schaffer F, Barkans J, Newman TM *et al.* Identification of interleukin-2 in human peripheral blood eosinophils. *Immunology* 1996; 87:155–61.
- 26 Lamkhioed B, Gouni AS, Aldebert D *et al.* Synthesis of type 1 (IFN gamma) and type 2 (IL-4, IL-5, and IL-10) cytokines by human eosinophils. *Ann NY Acad Sci* 1996; 796:203–8.
- 27 Lamkhioed B, Aldebert D, Gouni AS *et al.* Synthesis of cytokines by eosinophils and their regulation. *Int Arch Allergy Immunol* 1995; 107:122–3.
- 28 Grewe M, Czech W, Morita A *et al.* Human eosinophils produce biologically active IL-12: implications for control of T cell responses. *J Immunol* 1998; 161:415–20.
- 29 Baraniuk JN. Pathogenesis allergic rhinitis. *J Allergy Clin Immunol* 1997; 99:S763–72.
- 30 Ying S, Meng Q, Barata LT, Robinson DS, Durham SR, Kay AB. Associations between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression, and the infiltration of eosinophils, macrophages, and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* 1997; 158:5050–4.
- 31 Simon HU, Yousefi S, Schranz C, Schapowal A, Bachert C, Blaser K. Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. *J Immunol* 1997; 158:3902–8.
- 32 Frigas E, Motojima S, Gleich GH. The eosinophilic injury to the mucosa of the airways in the pathogenesis of bronchial asthma. *Eur Respir J* 1991; 13 (Suppl):123S–135S.
- 33 Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. *New Engl J Med* 1999; 340:197–9.