

Basophil recruitment and IL-4 production during human allergen-induced late asthma

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Background: Basophils represent an important source of inflammatory mediators and cytokines after IgE-dependent activation in human beings.

Objective: To assess the role of basophils in allergic asthma, we measured the number of basophils in the bronchial mucosa and their capacity to express IL-4 mRNA and protein during allergen-induced late asthmatic responses.

Methods: Fiberoptic bronchoscopic bronchial biopsies were obtained at 24 hours from sites of segmental bronchial allergen challenge and control sites in 19 patients with atopic asthma and 6 nonatopic healthy volunteers. Basophil numbers were assessed by immunohistochemistry through use of mAb 2D7. IL-4 mRNA-positive cells were detected through use of in situ hybridization and colocalized to basophils through use of sequential immunohistochemistry/in situ hybridization. IL-4 protein was detected and colocalized to basophils through use of dual immunohistochemistry.

Results: After allergen challenge, there was an increase in the median number of 2D7-positive basophils per square millimeter in the bronchial mucosa in patients with asthma (0.9 cells/mm² at baseline to 8.8 cells/mm² after challenge; $P = .002$), which also was significantly higher than what was seen in nonasthmatic controls ($P = .01$). Similarly, IL-4 mRNA-positive cells were increased at 24 hours in patients with asthma (1.4 to 14) in comparison with controls (0 to 0; $P = .02$). Colocalization studies revealed that 15% and 41% of the basophil population in patients with asthma after allergen-challenge expressed, respectively, IL-4 mRNA and protein. Conversely, 19% of IL-4 mRNA-positive cells and 72% of IL-4 protein-positive cells were accounted for by basophils.

Conclusion: After allergen provocation in sensitive patients with atopic asthma, basophils are recruited to the bronchial mucosa and express IL-4 mRNA and protein, which might contribute to local IgE synthesis and/or tissue eosinophilia or other aspects of allergic inflammation during late responses and ongoing asthma. (*J Allergy Clin Immunol* 2001;108:205-11.)

Key words: Asthma, late asthmatic responses, basophils, IL-4

Basophils express high-affinity IgE receptors and represent a potential major source of inflammatory mediators (eg, histamine, lipid mediators) and cytokines such as IL-4¹ after allergen-induced IgE-dependent activation. Previous studies have demonstrated infiltration by basophils of human allergen-induced late responses occurring in the skin, nose, and bronchi.²⁻⁶ However, these studies relied on morphologic criteria, metachromatic staining, and surface IgE expression. Differentiation from mast cells was achieved by the presence/absence of tryptase-positive staining. However, basophils also produce low levels of tryptase, and monocytes, eosinophils, and dendritic cells also express surface Fcε receptor I (FcεRI) and consequently might carry IgE on their cell surfaces. In 1995, through use of immunohistochemistry and immunogold electron microscopy, a murine IgG1κ mAb, 2D7, was identified that recognizes a specific determinant contained in the cytoplasmic granules of human basophils.⁷ Immunohistochemistry using 2D7 on microscopic sections of cutaneous biopsies obtained after intradermal allergen-challenge confirmed increases in basophil numbers during human late skin responses.⁸ Increases in basophils were also detected in sputum at 7 hours and 24 hours after allergen inhalation challenge through use of 2D7.⁹ An alternative basophil-specific marker, mAb BB1, has been used to detect basophil infiltration during late responses in the skin and bronchi.¹⁰⁻¹²

IL-4 is an archetypal "T_H2-type" cytokine that has been detected at the mRNA and protein level in bronchoalveolar lavage fluid and bronchial biopsies from patients with atopic asthma and during late asthmatic responses.¹³ IL-4 (as well as IL-13) is an important cofactor involved in the initiation of switching of B lymphocytes in favor of IgE production.¹⁴ IL-4 induces bronchial mucus production and upregulates the expression of vascular cell adhesion molecule 1 (VCAM-1) on vascular endothelium.¹⁵ For these reasons, it is likely that IL-4 plays an important role in the tissue eosinophilia and IgE production that characterize late responses and ongoing atopic asthma. Whereas a major cell source of mRNA for IL-4 during these responses has been identified as the T lymphocyte, it is clear from studies of human basophils derived from peripheral blood that the basophil represents an alternative source of IL-4 protein secretion.¹

In this study, we obtained bronchial biopsies 24 hours after bronchial segmental allergen provocation in patients with atopic asthma. Through use of immunohis-

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Abbreviations used

FcεRI:	Fcε receptor 1
PC ₂₀ :	Provocation concentration that caused a 20% decrease in FEV ₁
PEFR:	Peak expiratory flow rate
TBS:	Tris-buffered saline solution
VCAM-1:	Vascular cell adhesion molecule 1

tochemistry and in situ hybridization, we tested the hypothesis that basophils are recruited to the bronchi after allergen challenge and have the potential to express IL-4 mRNA and protein and contribute to the development of late asthmatic responses.

METHODS**Study design**

The study was performed with the approval of the Royal Brompton Hospital Ethics Committee, and all patients gave written informed consent. Each subject attended on a screening day when a history was taken, an examination and skin prick tests were performed, and a venous blood sample was taken for measurement of serum IgE antibody concentrations. Patients and normal volunteers returned to the hospital for overnight admission for the performance of fiberoptic bronchoscopy, segmental bronchial allergen provocation, and a repeat bronchoscopy after an interval of 24 hours.

Patients

Each of the 19 patients with asthma gave a history of asthma symptoms on relevant allergen exposure and was clinically stable. Baseline FEV₁ was greater than 80% predicted. Patients with asthma had increased bronchial responsiveness to inhaled methacholine (PC₂₀, <16 mg/mL, PC₂₀ being the provocation concentration of methacholine required to decrease FEV₁ by 20% from baseline). Their allergen sensitivity was confirmed by a positive skin prick test result (>5-mm skin weal) to either house dust mite (*Dermatophagoides pteronyssinus*) or grass pollen (*Phleum pratense*; Soluprick; ALK, Horsholm, Denmark) as well as increased serum allergen-specific IgE for the same allergen (CAP system, Pharmacia Diagnostics, Uppsala, Sweden). Inhaled glucocorticoid therapy had not been taken for at least 2 weeks, none of the subjects had received oral glucocorticoids for at least 6 months before bronchoscopy, and none had received immunotherapy during the previous 5 years. Smokers and exsmokers who had smoked more than 5 pack-years were specifically excluded. As a control group, we included 6 nonatopic healthy volunteers who had no symptoms suggestive of asthma or allergic disease, had normal methacholine bronchial reactivity (methacholine PC₂₀ >32 mg/mL), and had negative skin prick test results to a panel of 11 common aeroallergens, including pollens (tree, grass), molds (*Aspergillus*, *Alternaria*, *Cladosporium*), weeds (mugwort), animal extracts (cat, dog, horse), and house dust mite.

Bronchoscopy

All patients and controls were premedicated with nebulized albuterol (2.5 mg) and intravenous atropine (0.6 mg) and midazolam (5 to 10 mg) 10 minutes before the procedure. Oxygen was delivered throughout the procedure via nasal cannulae, and oxygen saturation was monitored by pulse oximetry. Topical anesthesia of the upper airways was achieved through use of 2% to 4% lidocaine solution. For subjects who underwent dual diluent/allergen chal-

lenge, the bronchoscope (Olympus BFP20; Olympus Corp, London, United Kingdom) was wedged in a lateral subsegment of the right middle lobe to undertake a control challenge with 5 mL of sterile saline solution prewarmed to 37°C. The bronchoscope was then passed into a subsegment of the lingula of the left lung, and allergen challenge was performed by instilling 5 mL of prewarmed saline solution containing 100 biological units of either *D pteronyssinus* or *P pratense* (Aquagen, ALK, Abello, Horsholm, Denmark). The allergen-challenged site was observed for 5 minutes, and in the absence of excessive local bronchoconstriction, a further 400 biological units of allergen was introduced in 5 mL of warmed saline solution. The subjects were observed closely, and peak flow values were recorded hourly throughout the day; the subjects were admitted overnight. A second bronchoscopy was performed with the same premedication at 24 hours after allergen challenge. Two bronchial biopsies were taken from each challenge site. For the remaining subjects, 2 baseline endobronchial biopsies were taken from either the carinae of the left lingular lobe or the right middle lobe. Allergen challenge was performed in either the right middle lobe or the lingula of the left lung—ie, in the site opposite that from which the baseline biopsies had been taken. A second bronchoscopy was performed 24 hours later, and 2 endobronchial biopsies were taken from the allergen-challenged site.^{16,17}

Bronchial biopsies

Bronchial biopsies were fixed in 4% paraformaldehyde/PBS solution for 2 hours, washed in 15% sucrose/PBS twice, embedded in OCT compound (BDH Merck, Lutterworth, United Kingdom), and snap-frozen in isopentane precooled in liquid nitrogen. Cryostat sections 6 μm thick were cut on polysine microscope slides (BDH Merck), dried overnight at 37°C, and stored at -80°C.¹⁸

Immunohistochemistry

Basophils were detected in bronchial biopsies through use of mAb 2D7. Sections were immersed in 0.6% hydrogen peroxide and methanol for 30 minutes to inhibit endogenous peroxidase activity. They were then washed twice in Tris-buffered saline solution (TBS) pH 7.6 before incubation with normal horse serum (Vectastain ABC kit, Vector Laboratories, Peterborough, United Kingdom) for 1 hour at room temperature. Sections were incubated at 4°C overnight with mAb 2D7 (0.65 μg/mL) or with the isotype-matched negative control (MOPC-31C, Sigma Chemical Company, St Louis, Mo). Sections from a formalin-fixed, paraffin-embedded blood clot that contained peripheral blood basophils were used as a positive control. After several washes with Tween 20 TBS (Sigma-Aldrich, Dorset, United Kingdom), sections were incubated with biotinylated horse antimouse IgG (Vector ABC kit) for 1 hour at room temperature. Slides were then incubated with Vectastain avidin biotin complex for 30 minutes at room temperature. Sections were monitored microscopically during incubation with the Vector diaminobenzidine substrate kit. Finally, slides were washed and mounted in Glycergel (Dako Ltd, Cambridge, United Kingdom).

Immunoreactive IL-4 protein was detected through use of a biotinylated rat antihuman IL-4 mAb (Pharmingen, BD, Cowley, Oxford, United Kingdom). Sections were permeabilized by washing in freshly prepared 0.1% saponin in PBS for 30 minutes. Sections were then treated with an avidin/biotin blocking kit (Vector Laboratories) to block nonspecific binding of biotin/avidin system reagents. Slides were incubated with avidin D blocking solution for 15 minutes, rinsed briefly in saponin/PBS, and then incubated for 15 minutes with the biotin blocking solution. Sections were incubated overnight at room temperature with biotinylated rat antihuman IL-4 mAb or biotinylated isotype-matched mAb of irrelevant specificity (Pharmingen) diluted in saponin/PBS containing 150 μL

rabbit serum per 10 mL PBS. Nasal biopsy sections (6 hours after allergen challenge) known to contain IL-4-expressing cells were used as a positive control.¹⁹ Slides were then washed with saponin/PBS and incubated for 30 minutes with biotinylated rabbit antirat IgG (Vector Laboratories) in saponin/PBS/rabbit serum.¹⁹ The sections were washed with saponin/PBS and incubated for 30 minutes with Vectastain ABC-AP (Vector Laboratories); next, they were washed and incubated for 20 minutes with fast blue BB salt or fast red TR salt (Sigma Aldrich). The slides were then washed in water and mounted in Glycergel. Double immunohistochemistry to detect the numbers of 2D7-positive cells expressing IL-4 immunoreactive protein was performed through use of mAb 2D7 followed by immunostaining for IL-4 protein, as described above. Mouse IgG1 isotype-matched negative control (for 2D7) (anti-*Aspergillus niger* glucose oxidase; Dako Ltd) and biotinylated rat IgG1 isotype-matched negative control (for IL-4) (Pharmingen) were included at the appropriate stages of double-staining.

In situ hybridization

Riboprobes, antisense and sense, were prepared from cDNA encoding human IL-4. cDNA was inserted into different pGEM vectors and linearized with appropriate enzymes before transcription. Transcription was performed in the presence of ³⁵S-uridine triphosphate and the appropriate T7 or SP6 RNA polymerase. For in situ hybridization, 6-μm sections were permeabilized with Triton X-100 (Sigma-Aldrich) and then digested with proteinase K (Sigma). To inhibit nonspecific binding of ³⁵S probes, sections were pretreated with iodoacetamide and N-ethylmaleimide followed by acetic anhydride/triethanolamine.²⁰ Sections were then hybridized with either antisense cytokine probes or (control) sense probes (with an identical sequence to mRNA), as described.²⁰ As a negative control, sections were treated with ribonuclease-A solution before the hybridization with antisense probes. For simultaneous in situ hybridization and immunohistology, sections were first immunostained through use of the 2D7 basophil marker, as described above; this was followed by in situ hybridization to detect IL-4 mRNA.

Quantification

2D7-positive basophils were detectable by a discrete intracellular granular staining pattern of cells. For in situ hybridization, hybridized signals for IL-4 mRNA were localized as a dense accumulation of silver grains in photographic emulsion overlying individual cells. Slides were counted blind in a random coded fashion through use of a Zeiss Axiolab microscope (Jena, Germany) fitted with an eyepiece graticule (0.202 mm² at a magnification of ×200). Subepithelial counts were expressed in numbers per square millimeter. The mean intraobserver coefficient of variation for repeat counts was 8%.

Statistical analysis

The Wilcoxon matched-pairs signed-rank test was used for within-group comparisons. Between-group comparisons were obtained through use of the 2-tailed Mann-Whitney *U* test. *P* values less than .05 were considered statistically significant. Statistical tests were performed through use of a standard computer package (Minitab Inc, State College, Pa).

RESULTS

Clinical data

Clinical data for the 19 atopic patients with asthma and the 6 nonatopic normal volunteers studied are shown in Table I. Twelve of the patients with asthma demon-

TABLE I. Clinical data for participants

	Patients with asthma (n = 19)	Normal controls (n = 6)
Age* (y)	30 (24,38)	22 (20,25)
Gender (male:female)	8:11	5:1
Total IgE* (IU/mL)	264 (120,470)	46 (12,177)
Allergen specific IgE* (IU/mL)	45 (28,101)	<0.35
Late-phase response* (% decrease in peak expiratory flow rate)	24.5 (19,33)	8
Methacholine PC ₂₀ (mg/mL)	3.4 (1.3,14.2)	>32

*Data are expressed as medians (interquartile ranges).

strated a greater than 20% fall in peak expiratory flow rate (PEFR) during the late period (3-24 hours) after allergen challenge, whereas none of the normal controls showed a decrease in peak flow rate greater than 10%.

Immunohistology: 2D7-positive basophils

Basophils were seen within the bronchial mucosa as discreet cells with granular cytoplasmic brown staining. In contrast, control sections stained with isotype control antibody from the same species were entirely negative. Positive control sections of blood clot that contained peripheral blood basophils confirmed 2D7 reactivity with basophils. In patients with asthma, median (interquartile range) numbers of 2D7-positive basophils per square millimeter within the bronchial mucosa were 0.9 (0 to 2.6) at baseline and 8.8 (2.5 to 12.9) 24 hours after segmental bronchial allergen provocation (*P* = .002). Corresponding numbers of basophils per square millimeter in normal nonasthmatic controls were 0.51 (0 to 1.3) and 0 (0 to 1.6; *P* = .9). Basophil numbers after allergen challenge were significantly higher for patients with asthma than normal controls (*P* = .01; Fig 1).

In situ hybridization

Specific hybridization signals were recognized as clear, dense deposits of silver grains in the photographic emulsion overlying cells expressing IL-4 mRNA. The specificity of the results obtained during hybridization with the antisense IL-4 Riboprobe was confirmed by the complete absence of hybridization signals in the control experiments using the IL-4 sense probe (with identical nucleotide sequence to IL-4 mRNA). Median (interquartile range) numbers of IL-4 mRNA-positive cells per square millimeter for patients with asthma were 1.4 (0 to 10.6) before and 14.0 (0 to 33.0) after allergen challenge (*P* = .02); corresponding values in normal subjects were 0 (0 to 2.7) before and 0 (0 to 0) after allergen challenge (*P* = .4). A comparison of IL-4 mRNA-positive cells after allergen challenge between patients with asthma and normal subjects was also statistically significant (*P* = .02; Fig 1).

Colocalization of IL-4 mRNA to basophils

Simultaneous immunohistology/in situ hybridization was performed on 7 biopsies obtained from the patients

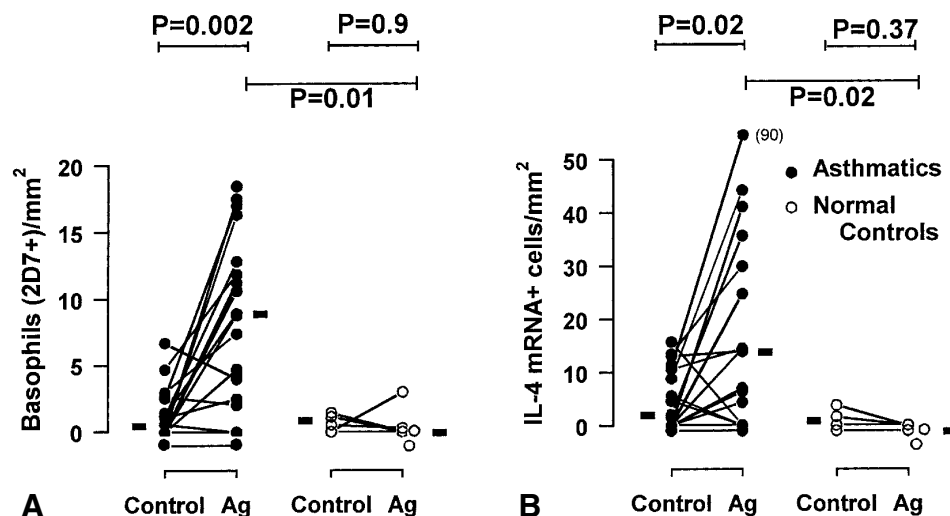


FIG 1. Immunohistochemistry for basophils (2D7; **A**) and in situ hybridization for IL-4 mRNA-positive cells (**B**) in the bronchial mucosa of asthmatic (●) and normal nonatopic control (○) subjects before and 24 hours after segmental allergen (Ag) challenge. Median values are represented by the horizontal bars. Within-group comparisons: Wilcoxon test. Between-group comparisons: Mann-Whitney *U* test.

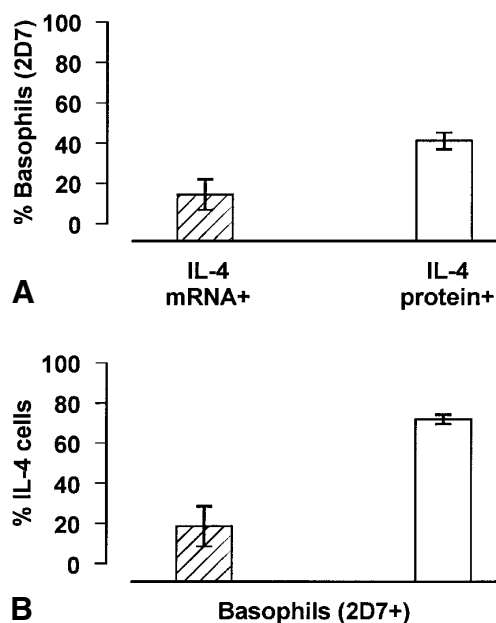


FIG 2. **A**, The percentages of basophils that express IL-4 mRNA (striped bar) and protein (open bar). **B**, The percentages of IL-4-positive cells (mRNA [striped bar] and protein [open bar]) that are basophils ($n = 7$).

with asthma in whom the number of IL-4 mRNA-positive cells and 2D7-positive cells was greater than 10 per square millimeter. This was carried out on biopsies taken 24 hours after allergen challenge to identify the propor-

tion of 2D7-positive basophils that expressed IL-4 mRNA (Fig 2, **A**) and, conversely, the contribution of basophils (%) to the total number of cells that expressed IL-4 mRNA during late responses (Fig 2, **B**). The mean ($\pm$ SEM) percentage number of 2D7-positive cells expressing IL-4 mRNA was 15% (8.8%). Conversely, 19% (10%) of IL-4 mRNA-positive cells were identified as basophils.

Colocalization of IL-4 protein

IL-4 protein staining demonstrated discreet cytoplasmic staining (red) of individual cells. Positive control sections consistently provided IL-4-positive expressing cells, whereas no staining was observed when isotype control antibody (negative control) was used. Double immunohistochemistry was performed through use of mAb 2D7 to detect basophils and an anti-IL-4 antibody to detect IL-4 protein product. The mean ($\pm$ SEM) percentage of 2D7-positive cells expressing IL-4 protein was 41% (4%), whereas 72% (2%) of immune-reactive IL-4 protein was colocalized to 2D7-positive basophils (Fig 2). Examples of individual and dual immunohistochemistry/in situ hybridization of bronchial mucosal sections for IL-4 mRNA and 2D7-positive specific staining for basophils are shown in Fig 3.

Interrelationship between basophils, IL-4, and the late response

Three of the 5 of 19 individuals who showed no basophils were also among the 6 of 19 who showed no IL-4 mRNA (Fig 1). Overall, there was no correlation between the number of IL-4 mRNA-positive cells and the number of 2D7-positive basophils. There was, however, a weak correlation between the changes in numbers

of IL-4 protein–positive cells and the changes in numbers of 2D7-positive basophils recruited to the bronchial mucosa during the late response ($r = 0.51$, $P > .05$). There was also no correlation between IL-4 mRNA–positive cells ($r = -0.05$, $P =$ not significant) or IL-4 protein positive cells ($r = -0.4$, $P < .1$) or basophil numbers ($r = 0.2$, $P =$ not significant) and the percent fall in PEFR during late responses after challenge.

DISCUSSION

We have shown that human allergen-induced late asthmatic responses are accompanied by increases in tissue basophils, a proportion of which express IL-4 mRNA and protein in the bronchial mucosa at 24 hours after challenge. Basophils recruited during late asthmatic responses therefore have the potential to contribute to the tissue eosinophilia—and possibly to the IgE synthesis—that characterize late responses and ongoing allergic asthma.

Basophil numbers were not increased in normal controls who also underwent allergen challenge. Had the increase in basophils in the patients with asthma been due to a nonspecific irritant effect of the provocation procedure itself or of the diluent, this would have been detected after challenge with the diluent control in both the patients with asthma and the normal nonatopic control subjects. The numbers of 2D7-positive basophils (9 per square millimeter) at the site of allergen challenge in this study are comparable to those of Macfarlane et al,¹² who used the basophil granule–specific mAb BB1 (~7.5 per square millimeter, a correction factor of 5 being used for the number of cells which in that study were expressed per millimeter of basement membrane length). Double immunohistochemistry and immunohistochemistry/in situ hybridization provided precise methods of identifying double-positive cells in the same rather than in serial sections. The discrepancy between the lower proportion of 2D7-positive basophils expressing IL-4 mRNA and the higher proportion expressing immunoreactive IL-4 protein might be explained, at least in part, by the ability of these cells to both synthesize IL-4 after allergenic stimulation (detectable at mRNA level) and store mature IL-4 protein, presumably within intracytoplasmic granules, before release on activation. In the present study, 19% of IL-4 mRNA–expressing cells were colocalized to 2D7-positive basophils. In a previous study, we reported that T lymphocytes accounted for 70% to 80% of cells expressing IL-4 mRNA,²¹ which is compatible with the present findings. In the former study, no IL-4 protein staining was attributable to T cells.²¹ IL-4 protein is synthesized and secreted, but not stored, by T cells, whereas basophils store IL-4 within their intracytoplasmic granules. Support for the latter assertion comes from in vitro studies of human basophils in which IL-4 mRNA expression by peripheral blood basophils increased to its peak level within 30 minutes during culture with anti-IgE and returned to baseline at 18 hours; in contrast, IL-4 protein remained at the peak level throughout the 18-hour culture.²² A time course of IL-4 mRNA

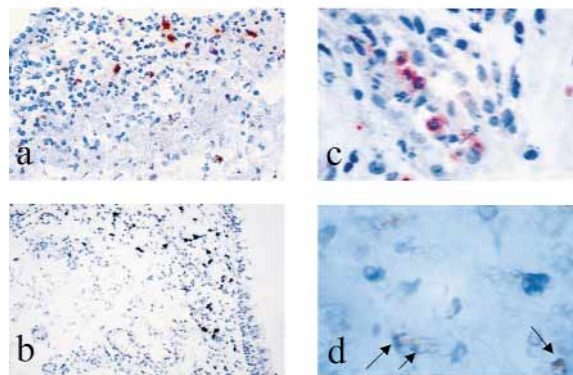


FIG 3. Sections of bronchial mucosa. **a**, 2D7-positive basophils are stained brown (background hematoxylin; $\times 40$). **b**, Cells positive for IL-4 mRNA are represented by discrete clusters of silver grains ($\times 20$). **c**, Cells positive for IL-4 protein are stained red ($\times 100$). **d**, IL-4 protein–positive/2D7-positive basophils are detected after double immunostaining for 2D7 (brown) and IL-4 protein (blue; $\times 100$).

and protein expression in the bronchi after allergen challenge might have provided support in vivo for this possibility, but this was not possible for ethical reasons.

A recent study of allergen-induced late cutaneous responses examined infiltrating basophils and other inflammatory cells.¹¹ Basophils were detected through use of an alternative, recently characterized basophil-specific marker, BB1. Basophil numbers were less numerous than eosinophils. In agreement with our findings pertaining to the bronchi, basophils were detectable at 24 hours in the skin after allergen challenge.¹² In that study, in which it was possible to examine a time course, basophils peaked at 24 hours whereas eosinophils peaked earlier, at 6 hours. In patients with atopic asthma, BB1-positive basophil numbers in the bronchial mucosa were 10-fold less than associated eosinophil and mast cell numbers.¹² Increases in BB1-positive basophils were observed after allergen challenge, which, allowing for the differences in expression of cell numbers, were comparable to those observed in the present study. Although a proportion of IL-4 mRNA (19%) and protein (41%) was colocalized to basophils, there was no significant correlation between IL-4 expression and basophil numbers. Similarly, there was no correlation between basophil numbers, IL-4 expression, and late falls in PEFR. This is not surprising, because the allergen was delivered into a local bronchial segment and local changes in airway caliber would not be reflected in overall changes of lung function.

In addition to histamine and lipid mediators such as leukotriene C₄,²³ human peripheral blood-derived basophils have been shown to secrete the chemokine macrophage inflammatory protein-1 α ²⁴ and the cytokines IL-4^{25–28} and IL-13^{29–31} after IgE-dependent stimulation. By use of flow cytometry, intracellular IL-4 was detected in unstimulated basophils from both atopic and nonatopic subjects, which indicated constitutive expression of IL-4 by basophils.³² Presumably, these cells in peripheral blood had not been stimulated by a specific antigen, whereas it is likely that the increases in IL-4

mRNA-positive basophils that we observed after allergen provocation in the bronchi represent activation of allergen-specific cells after allergen exposure.

IL-4 induces B-cell Ig switching in favor of IgE.³³ Although IL-4 is sufficient to initiate IgE transcription through the heavy chain (C ϵ) locus, with production of "sterile" nontranscribable ϵ germline transcripts, switch recombination and the expression of mature C ϵ transcripts (containing variable diversity joining sequences) require a second signal—ie, CD40 ligand. CD40 ligand is expressed on the surfaces of both activated T lymphocytes and basophils.^{34,35} Basophils cultured from umbilical cord blood both secrete IL-4 and express detectable levels of CD40L after cross-linking of Fc ϵ RI. When these activated basophils were added to normal human B cells, significant levels of IgE, IgG₄, and soluble CD23 were released. It seems likely that basophils represent a prominent early cell source during human allergic responses in providing both the IL-4 and the CD40L necessary for ongoing IgE synthesis by B lymphocytes. In addition, IL-4 might selectively increase VCAM-1 expression on the vascular endothelium. The cell surface counter ligand for VCAM-1 is VLA-4, which is largely confined to eosinophils and basophils.³⁶ Upregulation of VCAM-1 by IL-4 represents a mechanism for selective recruitment of eosinophils to sites of allergic reactions.

In summary, we have confirmed that after allergen exposure in sensitive patients with asthma there occurs a marked infiltration of basophils into the bronchial mucosa at 24 hours. Colocalization studies confirmed that basophils express IL-4 at the mRNA and protein level and thereby have the potential to contribute to local IgE and tissue eosinophilia during late responses and ongoing bronchial asthma.

Bronchial basophils and IL-4 expression represent potential targets for asthma therapy. For example, in patients with moderately severe asthma, nebulized soluble human IL-4 receptor demonstrated a marked steroid-sparing effect that persisted for 2 weeks after a single inhaled dose.³⁷ An alternative strategy might include the targeting of basophils, an important source of IL-4.

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