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Identification of Messenger RNA for IL-4 in Human Eosinophils with Granule Localization and Release of the Translated Product

Redwan Moqbel,* Sun Ying,* Julia Barkans,* Terence M. Newman,† Patrick Kimmitt,* Matthew Wakelin,* Luis Taborda-Barata,* Qiu Meng,* Christopher J. Corrigan,* Stephen R. Durham,* and A. Barry Kay2*

Human eosinophils are cytokine-producing cells that are prominent in IgE-dependent allergic tissue reactions. IL-4 promotes the development of the Th2-type phenotype in T cells and is an essential cofactor for IgE production by B cells. We detected mRNA for IL-4 by reverse transcription-PCR in blood eosinophils from atopic asthmatics. By specific ELISA, 108 -C 20 pg of development of the Th2-type phenotype in double immunocytochemistry on skin biopsies with eosinophil- and 11-4-specific mAb, 83.5 -C 3.5% of eosinophils were I1-4+. Eosinophils with Granule localization and Release Conversely, eosinophils accounted for 46.5

Materials and Methods
Isolation of peripheral blood eosinophils
Peripheral blood (100 ml) was obtained from mild atopic asthmatics not receiving oral corticosteroids (eosinophils >10% of the total leukocyte count) as well as from normal nonatopic healthy subjects. After dextran sedimentation of the erythrocytes, the granulocyte pellet was obtained by

**Declarations**

1 Abbreviations used in this paper: BAL, bronchoalveolar lavage; LPR, late phase reaction; ECP, eosinophil cationic protein; LDH, lactate dehydrogenase; TBS, Tris-buffered saline; RT, room temperature; APAAP, alkaline phosphatase-anti-alkaline phosphatase; DAB, diaminobenzidine; ISH, in situ hybridization; RT-PCR, reverse transcription-PCR; MBP, major basic protein.

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density centrifugation on Percoll. Eosinophils were purified by immunomagnetic selection using magnetic activated cell separator system (Becton Dickinson, Cowley, UK) as previously described (16). To eliminate contamination of the eosinophils with mononuclear cells, anti-CD14- and anti-CD3-coated micromagnetic beads (Lab Impex, Teddington, UK) were also added to the anti-CD16/granulocyte mixture. By negative selection, highly purified CD16+ eosinophils (>99% pure) depleted of negatively stained neutrophils (CD16−) and any contaminating mononuclear cells (CD14+*CD3−) were routinely obtained. The few contaminating cells were neutrophils.

The IL-4 content of blood eosinophils was estimated as follows. Freshly isolated and purified cells from atopic asthmatics were washed once by sedimentation (5 min, 120 × g) in PBS and resuspended in the same buffer at a concentration of 5 × 10⁶ cells/ml. Purified eosinophils from each donor were divided. One aliquot was disrupted with three cycles of freeze-thawing, and IL-4 was measured in the lysate using a specific ELISA (CLB, Amsterdam, The Netherlands) according to the manufacturer’s instructions. The other aliquot of cells was incubated with serum-treated Sephadex G-15 beads for 45 min at 37°C as previously described (17), and IL-4 was measured in the supernatant. The sensitivity of the assay was 2.3 pg/ml.

**Fractionation of human eosinophils**

The method for separation of granule-, plasma membrane- and cytosol-rich fractions of highly purified human eosinophils (99% pure) from atopic asthmatics has been described previously (18). Briefly, 5 × 10⁶ cells were sedimented by centrifugation at 240 × g for 5 min, and the pellet was resuspended in ice-cold 0.25 M sucrose buffer containing 1 mM EDTA, and 5 μg/ml each of leupeptin, aprotinin and tosyl-L-arginine methyl ester (TAME) (Sigma Chemical Co., Poole, UK) and repelted at 4°C. For homogenization, the cells were subjected to 8 to 12 passes through a high-precision ball bearing device (EMBL, Heidelberg, Germany) with a clearance of 11 pm (19).

**Immunocytochemistry**

**ICC/ISH.** The slides were left to incubate overnight at RT. After washing in TBS, a secondary layer, rabbit anti-mouse IgG (1/120), Dako, High Wycombe, UK, was applied to the slides (30 min, RT), which were then washed in TBS and incubated (30 min, RT) with a tertiary layer of a conjugate (Dako; 1/40). Sections were developed sequentially in Fast Red (1 mg/ml) for BMK-13 (a mouse anti-MBP) used to detect BMK-13 (mouse anti-MBP) used to determine the eosinophil phenotype (25). The slides were left to incubate overnight at RT. After washing in TBS, a secondary layer, rabbit anti-mouse IgG (1/40), Dako, High Wycombe, UK, was applied to the slides (30 min, RT), which were then washed in TBS and incubated (30 min, RT) with a tertiary layer of a mixture of streptavidin peroxidase (Amersham; 1/150) and mouse APAAP conjugate (Dako; 1/40). Sections were developed sequentially in Fast Red (an APAAP substrate) followed by DAB (a peroxidase substrate; Sigma Chemical Co.). IL-4-producing cells stained brown, eosinophils stained red, and eosinophils producing IL-4 stained reddish brown. Appropriate negative controls were included. These were TBS alone, an irrelevant biotinylated rat Ab of the same isotype, anti-IL-4 in the absence of the second layer, and second layer alone) were routinely included. The coefficient of repeated measurements (23) was 11.5 (95% confidence limits).

**Skin biopsies of EPR**

These biopsies were performed as previously described (24). Six atopic subjects consented with hand eczema. Positive cells stained red. Appropriate negative controls (TBS only, an irrelevant biotinylated rat Ab of the same isotype, anti-IL-4 in the absence of the second layer, and second layer alone) were routinely included. The coefficient of repeated measurements (23) was 11.5 (95% confidence limits).

**Immunolabeling for electron microscopy**

This was performed as previously described (18). Briefly, pelleted isolated eosinophils were fixed in freshly prepared formaldehyde (2% in PBS, 0.1 M, pH 7.2) for 2 h and embedded in Lowicryl K4M resin using the Balzers FSU 010 preparation infiltration procedure. Silver sections were cut and picked up onto thin bar (460 TB Hex) nickel grids. Before labeling, sections were blocked from biopsies, air-dried overnight, and then fixed in acetone for 10 min. Endogenous peroxidase was blocked using 1% H₂O₂ (plus 0.02% azide in TBS) for 30 min. After a brief wash in TBS, sections were incubated in 1% BSA (Sigma Chemical Co.) in TBS for 30 min. A mixture of the primary Ab was added consisting of 1/50 dilution of anti-human IL-4 mAAb (1:50) used to detect IL-4 product and a 1/30 dilution of BMK-13 (mouse anti-MBP) used to determine the eosinophil phenotype (25). The slides were left to incubate overnight at RT. After washing in TBS, a secondary layer, rabbit anti-mouse IgG (1/40), Dako, High Wycombe, UK, was applied to the slides (30 min, RT), which were then washed in TBS and incubated (30 min, RT) with a tertiary layer of a mixture of streptavidin peroxidase (Amersham; 1/150) and mouse APAAP conjugate (Dako; 1/40). Sections were developed sequentially (23) was 11.5 (95% confidence limits).
5 min, and reverse transcribed in a final volume of 40 µl. The reverse transcription mix contained 40 U of avian myeloblastosis virus reverse transcriptase (Pharmacia, Milton Keynes, UK); 1X PCR buffer (50 mM Tris-HCl [pH 8.3], 50 mM KCl, and 50 mM MgCl2); 0.6 mM each of dNTP, dATP, dCTP, and dGTP (Pharmacia); 200 ng of BSA (Sigma Chemical Co., Poole, UK); 5 µg poly(dT)20-28 (Pharmacia); and 32.4 U of RNA-guard (Pharmacia). The mix was incubated at RT for 10 min followed by 42°C for 1 h. Following reverse transcription, the enzyme was inactivated by heating the mix to 80°C for 10 min. The resulting cDNA was stored at −80°C until used.

**PCR amplification**

PCR was performed on 1/20 dilutions of cDNA from the reverse transcription reaction above. A 5-µl aliquot of this dilution was used in a 50-µl PCR reaction containing 2.5 U of Taq DNA polymerase (Applied Biosystems, Warrington, UK), 1X PCR buffer, 0.2 mM dNTP, 0.1% Triton X-100 (Sigma Chemical Co.), and 0.4 µM each of appropriate forward (5') and reverse (3') primers. The IL-4 primer sequences were 5'-CGGCAACTTT GACCCACGAAAAGTCGATA-3' (5' primer) and 5'-ACGTACTC TGTTGGTCTCTTCACAGGACAG-3' (3' primer), which span a 344-bp region of the IL-4 cDNA. This primer crosses a genomic intron, thus precluding amplification of the genomic sequence. Positive and negative controls were included in each set of reactions, and amplification of the housekeeping gene β-actin was employed as an endogenous control. The β-actin primers were 5'-AAGGCGCAGCCAGGAAGAAGAGT3'-3' (5' primer) and 5'-ACAGGACTCCATGCCACGAGG-3' (3' primer), which span a 479-bp region of the β-actin cDNA. Samples were overlaid with 50 µl of light mineral oil (Sigma Chemical Co.) and transferred to an thermal cycler (Omnigene, Hybaid, UK). After an initial denaturation step at 95°C for 5 min, the samples were subjected first to four cycles of heating to 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, followed by an additional 36 cycles of heating to 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. A final extension step was then performed by heating to 72°C for 7 min. The amplified products were visualized by electrophoresis on a 10-µl aliquot on a 2% ethidium bromide-stained agarose gel.

**Analysis of PCR-amplified products**

Southern dot blotting was employed to confirm the identity of PCR products. Aliquots (5 µl) of PCR samples were denatured by the addition of 100 µl of 0.4 M NaOH and 10 mM EDTA and boiling for 10 min. Samples were immediately cooled on ice and neutralized by the addition of an equal volume of 2 M ammonium acetate, pH 7.0, and applied to a nitrocellulose membrane (Hybond-N, Amersham) using a dot blottling apparatus (Bio-Rad, Watford, UK). The cDNA was cross-linked to the membrane using a Spectrolineker XL-1000 (Spectronics Corp., New York, NY). The membrane was prehybridized for 1 h at 55°C in 6X standard saline citrate (SSC), 10 mM EDTA (pH 8.0), 2X Denhardt’s solution, 0.5% SDS, and 100 µg/ml denatured salmon sperm DNA (Sigma Chemical Co.). An internal IL-4 oligonucleotide probe end labeled with [γ-32P]ATP (Amersham) using polynucleotide kinase (Promega, Southampton, UK) was added and allowed to hybridize for 2 h at 55°C. The sequence of the IL-4 probe was 5’-GTCCTTCTCATGGTGGCTGTAGAACTGCCG-3’. The membrane was then rinsed several times in 2X SSC-0.5% SDS at room temperature and washed twice in 2X SSC-0.5% SDS for 20 min at 55°C. Autoradiography was performed using Kodak X-Omat AR film (Eastman Kodak, Hemel Hempstead, Herts., UK).

**Fiberoptic bronchoscopy, BAL cells**

Bronchoscopy and BAL were performed in subjects with documented atopic asthma, and BAL cells were processed as previously described (26,27). Briefly, cytospins were prepared from BAL cells (at a concentration of 0.3 × 10³ cells/ml) using RNase-free slides coated with 0.1% poly-l-lysine (Sigma Chemical Co.) using a Shandon 2 cytospin device (Shandon Southern Instruments, Runcorn, UK). Samples were air-dried for 10 min, fixed in 4% paraformaldehyde in PBS (pH 7.4) for 30 min, and washed in 15% sucrose (Sigma Chemical Co.) in PBS. Slides were incubated at 37°C overnight, then stored at −80°C until used.

**Quantitation of numbers of cells expressing cytokine mRNA and phenotypic markers**

In BAL cytospins, at least two slides from each of the BAL preparations were stained with EG2 mAb and subsequently probed for expression of IL-4 mRNA. EG2+ cells were stained red, while hybrids between cytokine mRNA and CRNPs probes were located as dense collections of silver grains in the photographic emulsion overlaying individual cells. Two observers quantified the numbers of eosinophils and those showing positive IL-4 signals in 1000 BAL cells/slide independently using a Zeiss microscope (Germany) with bright and dark fields. In the skin biopsies, numbers were expressed as the mean number of biopsy cores using a computerized program (Apple IIE, Apple Computer, Cupertino, CA). The interobserver coefficient of variability for BAL and skin biopsy counts was <5%.

**Results**

**Peripheral blood eosinophils**

Using RT-PCR amplification under the stated conditions, mRNA encoding IL-4 was detected in highly purified (>99%) peripheral blood eosinophils from five of seven atopic asthmatics (Fig. 1). The identity of the amplified IL-4 cDNA was verified by Southern dot blotting using internal oligonucleotide probes (Fig. 2).

Using the APAAP technique, freshly prepared cytospins of eosinophils (>99% pure) were examined for IL-4 immunoreactivity using a monoclonal anti-human IL-4 Ab (Fig. 3a). Cytoplasm- or granule-associated IL-4 immunoreactivity was observed in 21.8 ± 5% of eosinophils from atopic asthmatics and 38.0 ± 13% of eosinophils from nonatopic healthy controls (n = 6 in each case; Fig. 4). Relevant negative controls (irrelevant biotinylated rat Ab of the same isotype and omission of the primary Ab) showed no immunoreactivity. Stained eosinophils consisted of eosinophils from subjects containing between 10% and 30% blood eosinophils. Slides were incubated at 37°C overnight, then stored at −80°C until used.

**Sequential immunocytochemical staining and in situ hybridization**

**ICC**. BAL cytospins or sections from skin biopsies were warmed to RT. ICC using EG2 Ab and the APAAP technique was performed as previously described (28–30). For negative control preparations, the primary Ab was replaced by either an irrelevant mouse Ig or TBS. BAL cytospins or skin biopsies were stained with EG2 or MBP, respectively, and then probed with an IL-4 riboprobe as described below.

**ISH**. After immunocytochemical staining, BAL cells or skin biopsy specimens were then permeabilized by immersion in 0.3% Triton X-100 in PBS for 10 min. After a brief wash in PBS, specimens were further permeabilized by exposure to proteinase K (Promega) solution (1 mg/ml in 20 mM Tris-HCl and 1 mM EDTA, pH 7.2) for 10 min at 37°C, the activity of which was terminated by immersion in 4% paraformaldehyde in PBS for 5 min. After a brief rinse in PBS, slides were air-dried. Radiolabeled (Sulphur-35, Amersham) riboprobes (sense and antisense) were prepared from IL-4 cDNA in pGEM-type vectors as previously described (26,28,29,31). As a positive control for IL-4 mRNA+ cells, cytospins from a peripheral blood T lymphocyte clone obtained from a patient with the hyper-IgE syndrome known to express IL-4 mRNA were prepared (27). For negative control preparations, slides were hybridized to sense probes and preincubated with RNAse A, then hybridized to antisense probes (27,30,31).

**Peripheral blood eosinophils**

Using RT-PCR amplification under the stated conditions, mRNA encoding IL-4 was detected in highly purified (>99%) peripheral blood eosinophils from five of seven atopic asthmatics (Fig. 1). The identity of the amplified IL-4 cDNA was verified by Southern dot blotting using internal oligonucleotide probes (Fig. 2).
FIGURE 1. RT-PCR of eosinophil mRNA using primers specific for β-actin (first lane) and IL-4 (second lane). Third lane, m.w. markers to confirm the predicted sizes of amplified segments of β-actin (479 bp) and IL-4 (344 bp). Fourth lane, IL-4 control cDNA amplified using the same primers. Fifth lane, PCR negative control (RNA only).

There was a smaller peak of activity detectable in fractions with a density range of 1.04 to 1.17 g/ml and corresponding to fractions containing the CD9 plasma membrane marker. No IL-4 was detected in the cytosolic fractions of the lowest (1.03–1.07 g/ml) density containing the LDH marker.

Immunogold labeling of eosinophils with an anti-IL-4 mAb showed gold particles in association with eosinophil secretory granules, particularly the crystalline granule cores, with minimal labeling of the cytoplasm or nucleus. The amount of granule labeling was greater than that observed in control experiments using a biotinylated normal rat IgG or secondary streptavidin-gold only, where few gold particles of uniform distribution were seen (Fig. 6).

Bronchoalveolar lavage

Using sequential ICC and ISH, the expression of mRNA encoding IL-4 in EG2-immunoreactive eosinophils was determined using BAL cells from baseline (unchallenged) atopic asthmatics (Fig. 3). BAL contained a mean of 7.7 ± 1.4% EG2+ eosinophils (n = 7). Of the EG2+ cells, 1.6 ± 0.5% coexpressed IL-4 mRNA.

Cutaneous allergen-induced LPR

Sequential ICC and ISH were performed as described for BAL, except that eosinophils were identified by immunoreactivity with anti-MBP mAb (BMK-13). Skin biopsies obtained 6 h after allergen challenge of sensitized atopic subjects (n = 6) contained a mean of 26.7 eosinophils/mm², of which 22.4 ± 3.3% coexpressed IL-4 mRNA, while 52.7 ± 4.4% of cells expressing IL-4 mRNA were MBP+ (Table II and Fig. 3). IL-4 protein was also detected by a double immunostaining method in additional sections from the same biopsies (Table III and Fig. 3). A mean of 83.5% ± 3.5% of all eosinophils counted (MBP+) were IL-4+, while 46.5 ± 3.9% of IL-4+ cells were identified as MBP+ eosinophils. There was an approximate association between IL-4 mRNA and protein expression especially when the percentages of eosinophils expressing IL-4 mRNA and protein (third columns of Tables II and III) were compared (r = 0.54).

Discussion

We have demonstrated, using several approaches, that human eosinophils express mRNA encoding IL-4, store translated IL-4 protein in association with secretory granules, and release IL-4 in vitro following an appropriate stimulus (serum-coated particles). These findings are of clear relevance to the pathogenesis of allergic inflammation.

The detection of mRNA encoding IL-4 by RT-PCR in highly purified peripheral blood eosinophils from a majority of atopic asthmatics suggests that this cytokine is constitutively expressed in at least some of these patients in eosinophils. Contamination of eosinophils in these experiments was minimized by removal of CD16+ neutrophils, CD3+ T lymphocytes, and CD14+ monocytes. This procedure resulted in >99% purity of eosinophils obtained by the immunomagnetic microbead technique. Furthermore, we were unable to demonstrate IL-4 mRNA by RT-PCR analysis of similar small numbers of contaminating cells under the same experimental conditions.

To demonstrate unequivocally the presence of IL-4 mRNA in eosinophils, we used ISH combined with ICC to identify the cell phenotype. We have been able to present clear evidence that eosinophils express mRNA encoding IL-4 (BAL cells from unchallenged atopic asthmatics) and both IL-4 mRNA and protein (allergen-induced cutaneous LPR; Fig. 3 and Tables II and III). In the latter case, allergen challenge of skin was associated with substantial infiltration with eosinophils, high percentages of which expressed IL-4 mRNA and immunoreactivity (in the case of IL-4 protein, substantially higher than the percentages of positive cells in the peripheral blood). Since, as we have shown previously (1, 24), dilute challenge control skin sites contain very few eosinophils, this observation is compatible with the hypothesis that recruitment of eosinophils to sites of allergic inflammation is associated with increased IL-4 immunoreactivity of the infiltrating cells. We are currently evaluating the possible effects of agents previously shown to modulate eosinophil surface marker expression (33) or eosinophil expression of mRNA encoding cytokines other than IL-4 (34, 35) on IL-4 mRNA expression by eosinophils in vitro.
Immunocytochemical staining of peripheral blood eosinophils indicated that IL-4 immunoreactivity was detectable in a proportion of these cells, principally associated with the granules (Fig. 3). This was confirmed by detection of IL-4 in eosinophils disrupted by repetitive freeze-thawing (Table I). The amount of IL-4 in these cells was substantial (mean, 108 pg/10⁶ cells) and within the
physiologically relevant range. Similar percentages of eosinophils from both atopic asthmatics and normal controls showed IL-4 immunoreactivity, while in some subjects no immunoreactivity was observed (Fig. 4). These observations further validate the ICC technique in the sense that they clearly show that the Ab used in this technique did not adhere nonspecifically to the eosinophil surface. The demonstration of mRNA in eosinophils and the supernatants assayed for IL-4 of eosinophils stimulated with serum-coated particles suggested that eosinophils may represent an important repository for rapidly mobilizable IL-4, which may maintain a local environment conducive to the development of Th2-type T lymphocytes and IgE synthesis.

The granule-associated nature of IL-4 staining in peripheral blood eosinophils was confirmed by the cell fractionation technique employed here (Fig. 5). We previously used this method to identify GM-CSF localization to the eosinophil granule compartment (25). The technique permits the collection of subcellular fractions and separation into distinct subcellular compartments, i.e., secondary crystalloid granules, plasma membrane, and cytosol. The clear coelution of IL-4 with eosinophil granule markers, with negligible amounts found in association with plasma membrane (CD9⁺), and its absence in the cytosol confirmed the association of this cytokine with the eosinophil crystalloid granule. How far this reflects storage of IL-4 following de novo synthesis, as opposed to uptake by endocytosis via IL-4 receptors on eosinophils (36), remains to be determined. The demonstration of mRNA in eosinophils, both ex vivo and in vitro, is, however, compatible with the hypothesis that the intracellular protein detected is derived at least partly from de novo synthesis of IL-4.

Immunogold labeling of intact eosinophils examined under electron microscopy (Fig. 6) showed gold particles in association principally with the crystalline core of the eosinophil granule, thus providing further confirmation of the presence of IL-4 in human eosinophils and its close association with the crystalloid granules (Fig. 6). The immunogold labeling method was also used to confirm the association between eosinophil-derived GM-CSF and the crystalloid granules of these cells (18). Others have described the presence of TNF-α and IL-5 in association with eosinophil granules in patients with blood eosinophilia (15, 37).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Whole Cell Lysates</th>
<th>Supernatants from Serum-Coated Beads</th>
</tr>
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<tr>
<td>Subject 1</td>
<td>124 pg/10⁶ cells</td>
<td>32 pg/10⁶ cells</td>
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<tr>
<td>Subject 2</td>
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<tr>
<td>Subject 6</td>
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<td>61 pg/10⁶ cells</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>108 ± 20</td>
<td>34 ± 6</td>
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</table>

* Purified cells from each individual donor were divided and either disrupted with three cycles of freeze-thawing (whole cell lysates) or incubated with serum-treated Sephadex G-15 beads (or medium alone) for 45 min at 37°C, centrifuged, and the supernatants assayed for IL-4.

* Supernatants from cells incubated with medium alone had IL-4 concentrations of <5 pg/10⁶ cells.

**Figure 4.** Percentages of peripheral blood eosinophils obtained from atopic asthmatics and nonatopic healthy controls showing immunoreactivity to IL-4 using the anti-IL-4 mAb and the APAAP technique.

**Figure 5.** Fractionation profile of human eosinophils showing the elution of ECP, CD9, and LDH together with IL-4 immunoreactivity, as determined by ELISA and dot blot analysis. Eosinophil subcellular fractions were separated on the basis of their density (g/ml) on Nycodenz gradient.

**Table 1.** IL-4 immunoreactivity in whole blood eosinophils and in the supernatants of eosinophils stimulated with serum-coated particles.
FIGURE 6. Immunogold labeling of Lowicryl-embedded intact purified human eosinophils fixed with 2% formaldehyde. The sections were not counterstained, allowing the gold-labeled granules to be clearly observed. a, Rat anti-human IL-4 labeling (magnification, ×15,500); b, high magnification of a (magnification, ×34,000) to show distribution of gold particles over the granules; c, rat IgG control (magnification, ×15,500); d, secondary Ab gold only control (magnification, ×15,500).
Eosinophils were found to express IL-4 receptors (36). IL-4 may have been expressed on human eosinophils, but not neutrophils (40). IL-4 was chemotactic for eosinophils (but not neutrophils) observed in human eosinophils (45), would appear to synthesize and secrete more IL-4 than basophils (48). The precise bioavailability and bioactivity of intracellular immunolocalization of IL-4 in normal peripheral blood eosinophils (45), and in human peripheral blood eosinophils. J. Allergy Clin. Immunol. 95:342 (Abstr. 806).

Table II. MBP+ eosinophils expressing IL-4 mRNA in skin biopsies from atopic subjects 6 h after allergen challenge

<table>
<thead>
<tr>
<th>Subject</th>
<th>MBP+/IL-4- (cells/mm2)</th>
<th>MBP+/IL-4+ (cells/mm2)</th>
<th>% Eos/IL-4+</th>
<th>MBP+/IL-4+ (cells/mm2)</th>
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<td>± 1.4</td>
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Table III. MBP+ eosinophils expressing IL-4 protein in skin biopsies from atopic subjects 6 h after allergen challenge

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<th>Subject</th>
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<th>MBP+/IL-4+ (+ve/mm2)</th>
<th>% Eos/IL-4+</th>
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The present finding adds to previously established links between eosinophils and IL-4. For example, Dubois et al. (38) reported that IL-4 was chemotactic for eosinophils (but not neutrophils) obtained from patients with atopic dermatitis. In a preliminary report, eosinophils were found to express IL-4 receptors (36). IL-4 may also induce selective eosinophil infiltration in allergic inflammatory reactions indirectly by up-regulating the expression of VCAM-1 on endothelial cells (39), since VLA-4, the ligand for VCAM-1, is expressed on human eosinophils, but not neutrophils (40).

In addition to T cells, IL-4 is also a product of human leukemic and activated normal basophils (41) as well as mast cell lines (42). IL-4 has been immunolocalized in human mast cells in nasal mucosa (43). Human basophils, with or without stimulation (44, 45), would appear to synthesize and secrete more IL-4 than eosinophils (46). It has been suggested that basophil-derived IL-4 is synthesized de novo and secreted, but not stored, after IgE-dependent stimulation (45, 47), although others have demonstrated intracellular immunolocalization of IL-4 in normal peripheral blood basophils (48). The precise bioavailability and bioactivity of inflammatory cell-derived IL-4 compared with those of T cell production are unknown and difficult to document. However, the presence of this cytokine in a stored granule-associated form, at least in the eosinophil, suggests that this cytokine may exert its effect on the inflammatory reaction possibly via mechanisms involving juxtacrine intracellular signaling (49).

In conclusion, we have provided several lines of evidence suggesting that human eosinophils can synthesize IL-4 and store it in association with their crystalloid granules. Furthermore, we have shown that IL-4 is released from highly purified eosinophils following an appropriate stimulus. Thus, eosinophils have the potential to participate in allergic inflammatory reactions both by promoting IgE synthesis and by maintaining the Th2-type cytokine environment.

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References


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