

IL-9 expression by human eosinophils: Regulation by IL-1 β and TNF- α

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Background: IL-9 is a pleiotropic cytokine that exhibits biologic activity on cells of diverse hemopoietic lineage. IL-9 stimulates the proliferation of activated T cells, enhances the production of IgE from B cells, and promotes the proliferation and differentiation of mast cells and hematopoietic progenitors. **Objective:** In this study we evaluated the expression of IL-9 messenger (m)RNA and protein by human peripheral blood eosinophils. We also investigated the role of IL-1 β and TNF- α in the release of IL-9 from human peripheral blood eosinophils.

Methods: RT-PCR, in situ hybridization, and immunocytochemistry were used to investigate the presence of IL-9 mRNA and protein in human peripheral blood eosinophils from asthmatic patients and normal control subjects. Furthermore, biologic assay was used to investigate the release of IL-9 protein from IL-1 β - or TNF- α -stimulated eosinophils in vitro.

Results: RT-PCR analysis showed the presence of IL-9 mRNA in human peripheral blood eosinophil RNA preparations from subjects with atopic asthma, as well as in the eosinophil-differentiated HL-60 cell line. By using in situ hybridization, a significant difference ($P < .01$) in IL-9 mRNA expression was detected in human peripheral blood eosinophils freshly isolated from asthmatic subjects compared with those isolated from normal control subjects. Furthermore, the percentage of IL-9 immunoreactive eosinophils from asthmatic patients was increased compared with that found in normal control subjects ($P < .01$). We also demonstrate that cultured human peripheral blood eosinophils from asthmatic subjects synthesize and release IL-9 protein, which is upregulated on stimulation with TNF- α and IL-1 β .

Conclusion: Human eosinophils express biologically active IL-9, which suggests that these cells may influence the recruitment and activation of effector cells linked to the pathogenesis of allergic disease. These observations provide further evidence for the role of eosinophils in regulating airway immune responses. (*J Allergy Clin Immunol* 2000;106:460-6.)

Key words: Eosinophils, IL-9, regulation, IL-1 β , TNF- α

Abbreviations used

DMEM: Dulbecco's modified Eagle's medium

SSC: Standard saline citrate

TBS: TRIS-HCl-buffered isotonic saline, pH 7.6

Asthma is a chronic inflammatory disease of the bronchial airways that is clinically characterized by airways obstruction, enhanced bronchial responsiveness, and airways inflammation. The inflammatory component of this disease includes an increased number of activated T lymphocytes, mast cells, and eosinophils within the airway lumen and bronchial submucosa.¹⁻³ Considerable evidence from human and animal studies indicate that CD4⁺ T cells are the predominant cell type involved in the regulation of airways inflammation through the expression of T_H2-type cytokines.¹⁻² Moreover, eosinophils are thought to contribute to the physiologic changes and alterations in airway structure that characterize asthma by their release of cytotoxic proteins, reactive oxygen metabolites, and proinflammatory and profibrotic cytokines.³⁻⁴

IL-9 is a T_H2-type cytokine secreted by activated CD4⁺ T cells.⁵ It has been shown to promote the proliferation and differentiation of mast cells and hematopoietic progenitors to stimulate the proliferation of activated T cells and to enhance the production of immunoglobulins by B cells.⁶ A close association between the IL9 gene and bronchial-hyperresponsiveness has been suggested,⁷ and selective overexpression of the IL9 gene within the lungs of transgenic mice results in massive airways inflammation with eosinophils and lymphocytes as predominant infiltrating cells.⁸⁻⁹ On the basis of these findings, a potential role for IL-9 in asthma has been suggested.¹⁰

Because of the proposed central role of eosinophils in asthma and atopic disease, there is considerable interest to determine how these cells and their products contribute to the altered airway physiology and the mechanisms that mediate the expression of genes that promote eosinophilia, such as IL9.⁸⁻¹¹ In this study we investigated whether human eosinophils express IL-9 and if so, what factors are involved in regulating this expression. We demonstrate that human eosinophils from asthmatic subjects and normal control subjects express messenger

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Supported by the Medical Research Council of Canada and Magainin Pharmaceuticals, Inc.

Received for publication Feb 22, 2000; revised June 7, 2000; accepted for publication June 8, 2000.

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0091-6749/2000 \$12.00 + 0 1/1/109172

doi:10.1067/mai.2000.109172

(m)RNA coding for IL-9 protein. By using immunocytochemistry, a high level of IL-9 protein expression was demonstrated in eosinophils from asthmatic subjects compared with those from normal control subjects. We also show that stimulation of human peripheral blood eosinophils with TNF- α and IL-1 β results in an increased release of this cytokine.

METHODS

Reagents and antibodies

Goat polyclonal affinity-purified anti-IL-9 was obtained from Santa Cruz Biotechnology (Santa Cruz, Calif), and goat IgG was obtained from Sigma (Oakville, Ontario). Biotinylated rabbit anti-goat Ig, streptavidin-conjugated alkaline phosphatase, and Fast red were obtained from Dako (Dakopatt, Denmark). FBS was obtained from Hyclone Laboratories (Logan, Utah). Recombinant cytokines TNF- α and IL-1 β were purchased from R&D Systems (Minneapolis, Minn). Ficoll-Hypaque was obtained from Pharmacia (Uppsala, Sweden). Anti-CD16 and anti-CD3 magnetic bead mAbs were from Miltenyi Biotec (Auburn, Calif). Super-Script reverse transcriptase, Trizol, Ampli-Taq polymerase, antibiotics, Dulbecco's modified Eagle's medium (DMEM), G418, and deoxyribonucleoside triphosphate were purchased from Gibco BRL (Gaithersburg, Md). Tritiated thymidine was obtained from Amersham Life Science (Baie d'Urfe, Quebec, Canada).

Subjects

This study was approved by the Ethics Committee of the Montreal Chest Hospital, Montreal, Quebec. Eight patients with asthma, as defined by the American Thoracic Society, were included in this study.¹² All asthmatic subjects were atopic to one or more of the tested panel of allergens. The asthmatic patients ($n = 8$; 4 male subjects) exhibited mild-to-moderate asthma (mean FEV₁ percent predicted, 77%; range, 62%-67%). Eight nonatopic, nonasthmatic, control subjects with negative skin test responses and normal spirometry results were also studied. Patients had not received inhaled or systemic corticosteroids in the last 3 months and were not receiving any medications other than β -agonists. Subjects who had upper respiratory tract infections within the last month were excluded from the study.

Isolation of eosinophils from peripheral human blood

Blood was collected into sterile heparinized syringes from the peripheral vein of asthmatic and nonatopic, nonasthmatic, volunteer donors who gave informed consent. After the erythrocytes were sedimented with dextran for 45 minutes at room temperature, the granulocyte fraction was obtained by centrifugation through a cushion of Ficoll-Hypaque (1.77 g/mL) of the buffy coat at 350g for 30 minutes. After the hypotonic lysis of residual erythrocytes, eosinophils were separated from neutrophils by using negative immunomagnetic selection with a magnetic cell separation column, as previously described.¹³ Briefly, the erythrocyte-depleted granulocyte pellet was incubated for 45 minutes at 4°C with anti-CD16 mAb bound to immunomagnetic beads. When the mixture was applied to a steel wire column in a strong magnetic field, the CD16⁺ neutrophils were retained, whereas the CD16⁻ eosinophils were highly purified in the fraction that flowed through the column. Contaminating T lymphocytes and monocytes were further removed by incubating the CD16⁻ fraction for another 15 minutes with saturating concentrations of anti-CD3 magnetic beads. Cytofluorescence slides of the eosinophils stained with Wright's Giemsa stain showed that the purity of the isolated eosinophils was greater than 98% $\pm$ 1.5%. The viability of the cells was greater than 98%, as determined by trypan blue dye exclusion.

RT-PCR analysis

Total cellular RNA was extracted from highly purified eosinophils and an eosinophil-differentiated HL-60 cell line by using the Trizol method (Gibco BRL). Reverse transcription was performed by using 2 μ g of total RNA from each patient in a first-strand complementary (c)DNA synthesis reaction with Super-Script reverse transcriptase, as recommended by the supplier (Gibco BRL). PCR was performed by adding 1 μ L of the RT product into 50 μ L of total volume reaction containing 1 $\times$ buffer, 200 μ mol of each deoxyribonucleoside triphosphate, 20 pmol of each oligonucleotide primer, and 0.2 units of Ampli-Taq polymerase. Oligonucleotides specific for IL-9 sequences on either side of a splice junction were used in the PCR reaction to preclude amplification of possible contaminating genomic DNA. Oligonucleotide primers and probes synthesized on the basis of the entire coding region of the human IL-9 (GeneBank accession No. M86593 M55519)¹⁴ were as follows: 5'-primer, 5'-GCAACATCAGTTCTGGCCAC-3'; 3'-primer, 5'-TGCTTCCAGGCTCCCCAGA-3'. The PCR (35 cycles) was carried out in a thermal cycler (PTC100, MJ Research Inc). Each cycle consisted of incubations at 94°C (1 minute) for denaturation, 62°C (2 minutes) for annealing, and 72°C (1 minute, 30 seconds) for primer extension. The initial denaturation period was 5 minutes, and the final extension was 10 minutes. Amplified products were analyzed by means of DNA gel electrophoresis in 2% agarose visualized by ethidium bromide staining.

Cytospin preparations

Cytospin slides were prepared from peripheral blood eosinophils, fixed in 4% paraformaldehyde for 20 minutes at room temperature, and washed with 0.05 mol/L TRIS-HCl-buffered isotonic saline, pH 7.6 (TBS). After drying, the slides were stored at -80°C before immunocytochemistry or in situ hybridization.

Preparation of riboprobes

cDNA probe coding for IL-9 was inserted into pGEM vectors. The vector containing IL-9 cDNA was linearized with the appropriate enzymes and transcribed in vitro with either SP6 and T7 polymerases in the presence of sulfur 35 (³⁵S)-labeled uridine triphosphate to generate sense and antisense riboprobes, as recommended by the supplier. The percentage of the incorporation of the radioisotopes was approximately 40% to 50%.

In situ hybridization

In situ hybridization was performed as previously described.¹⁵ Briefly, cytospin preparations were first permeabilized by immersion in 0.3% Triton X-100 in PBS for 10 minutes, followed by exposure to proteinase K (1 μ g/mL in 20 mmol/L TRIS-HCl and 1 mmol/L EDTA, pH 7.2) for 30 minutes at 37°C. The slides were then prehybridized with 50% formamide in 2 $\times$ standard saline citrate (SSC) for 15 minutes at 37°C. Hybridization was performed with ³⁵S uridine triphosphate-labeled riboprobes (either antisense or sense) for 16 hours at 42°C. Posthybridization washings were done with SSC solutions (4 $\times$ SSC and 0.1 $\times$ SSC) followed by ribonuclease A treatment to remove unhybridized single-stranded RNA. The preparations were dehydrated, immersed in Amersham LM-2 emulsion fluid, and then subjected to autoradiography for 18 days. The autoradiograms were developed in Kodak D-19 developer, fixed, and subsequently counterstained with hematoxylin. As a negative control, sections were hybridized with the sense probe or were pretreated with ribonuclease A before hybridization with the antisense probe.

Immunocytochemistry

The cytospin preparations of purified eosinophils from asthmatic or normal donors were washed with TBS. After saturation for 15

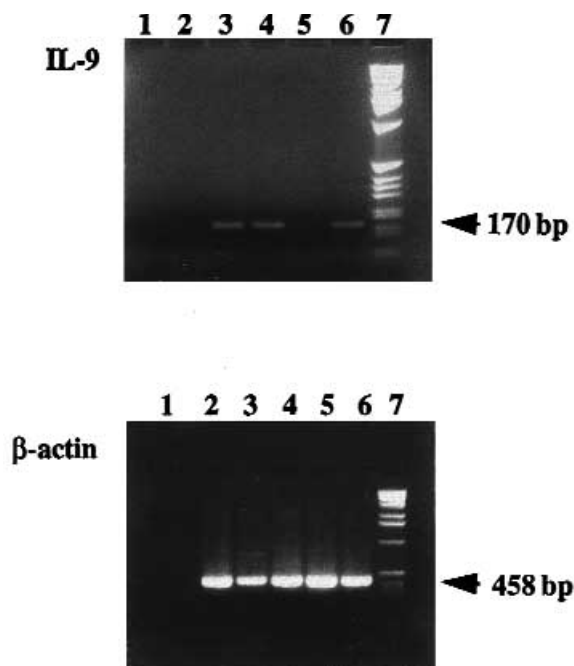


FIG 1. Detection of IL-9 transcripts in human eosinophil preparations. RNA preparation from human peripheral blood eosinophils (asthmatic subjects, lanes 2 and 3; normal control subjects, lanes 4 and 5) and eosinophil-differentiated HL-60 cells (lane 6) showed an IL-9-specific amplified fragment. Lane 1 corresponds to a PCR tube performed without cDNA. Lane 7 corresponds to a 1-kb DNA ladder molecular weight. β -Actin was used as control. Total eosinophil RNA was isolated, and first-strand cDNA synthesis was performed. Human IL-9 was amplified by using PCR and IL-9-specific primers.

minutes with universal blocking solution (Dako), cells were incubated with goat polyclonal anti-IL-9 or goat IgG (both at 1 μ g/mL) overnight at 4°C. After a washing step, biotinylated rabbit anti-goat diluted 1:200 was added for 30 minutes at 37°C. After 3 washes with TBS, the preparations were incubated with streptavidin-conjugated alkaline phosphatase diluted 1:200 for 1 hour at room temperature. Slides were washed with TBS and developed with Fast red followed by counterstaining with Mayer's hematoxylin.

Cell line and culture conditions

The human cell line HL-60 clone 15 was provided by ATCC (Rockville, Md). Cells were cultured at 37°C in humidified 5% CO₂ in RPMI-1640 medium supplemented with 10% heat-inactivated FBS and antibiotics (100 U/mL penicillin and 100 μ g/mL streptomycin). Differentiation toward eosinophils was performed as described previously.¹⁶ Briefly, cells were grown in RPMI-1640 and 10% FBS in the presence of butyric acid (0.3 mmol/L) for 7 days to generate eosinophils.

Eosinophil stimulation and IL-9 quantification

Freshly isolated eosinophils (2×10^6 /mL) were incubated at 37°C in humidified 5% CO₂ in RPMI-1640 medium supplemented with 10% heat-inactivated FBS and antibiotics for 18 hours at 37°C with either IL-1 β , TNF- α , or both at a final concentration of 20 ng/mL. This concentration was determined by a dose response in

preliminary experiments. After culture, supernatants were removed, clarified by centrifugation, and stored at -80°C until analysis.

Generation of cell lines

TS1 is an IL-9-dependent murine T-cell line.¹⁷ It was maintained in DMEM containing 10% FBS in the presence of 25 ng/mL murine IL-9. TS1 cells stably expressing human IL-9R (RA3 cells) were generated by electroporation and genitacin (G418) selection. Briefly, 3×10^6 cells were resuspended in 0.3 mL of RPMI-1640 containing 10 μ g of the appropriate plasmid pulsed (260 V/1000 μ F) in a GenePulse II electroporator (Bio-Rad) and resuspended in growth medium. After 24 hours, cells were selected in 1 mg/mL G418 for approximately 14 days, at which time mock-transfected cells died. Selected cells were maintained in growth medium supplemented with 25 ng/mL murine IL-9 and 0.4 mg/mL G418. Expression of human IL-9 receptor was periodically assessed by means of Western blotting.

Cell proliferation assay

Supernatants from eosinophil-induced cell proliferation were obtained as follows. RA3 cells were washed 3 times with PBS and resuspended in DMEM and 10% FBS. Then cells were seeded at 10^3 /200 μ L in 96-well microplates and grown in the presence of eosinophil supernatants without growth factors or various concentrations of recombinant human IL-9. Cells cultured with complete DMEM and 10% FBS alone were used as controls. After incubation at 37°C for 3 days, 1 μ Ci of tritiated thymidine (specific activity, 5 μ Ci/mmol) was added per well, and the wells were further incubated for 4 hours at 37°C and then harvested. The incorporated tritiated thymidine was determined by using a liquid scintillation counter (Top Count, Packard). For controlling the specificity of the proliferation, the TS1 cell line (nontransfected cells) was also cultured in the presence of eosinophil supernatants or different concentrations of human recombinant IL-9. The rate of proliferation was assessed by determining the ratio between the number of treated and untreated cells (percentage of control).

Statistics

Data are presented as means $\pm$ SD. Statistical significance was determined by using the Mann-Whitney *U* test. Probability (*P*) values of less than .05 were considered statistically significant. Statistical analyses were performed with the use of a standard computer package (Systat version 7.0; Systat, Evanston, Ill).

RESULTS

Detection of mRNA encoding IL-9 on human eosinophils

RNA preparations from highly purified eosinophils were first analyzed by using RT-PCR to determine whether eosinophils express IL-9 mRNA. As shown in Fig 1, a specific band at 170 bp was detected at the expected size from both asthmatic and normal control samples (lanes 2 and 3 and lanes 4 and 5, respectively), as well as the eosinophil-differentiated HL60 cell line, which was used as a positive control (lane 6). In situ hybridization was then performed to ascertain whether human peripheral blood eosinophils could express IL-9 mRNA. In Fig 2, A, peripheral blood eosinophils from asthmatic subjects show a positive signal with the ³⁵S-labeled antisense IL-9 riboprobe. No cells were labeled with the sense riboprobe (data not shown). The percentage of positive cells was sig-

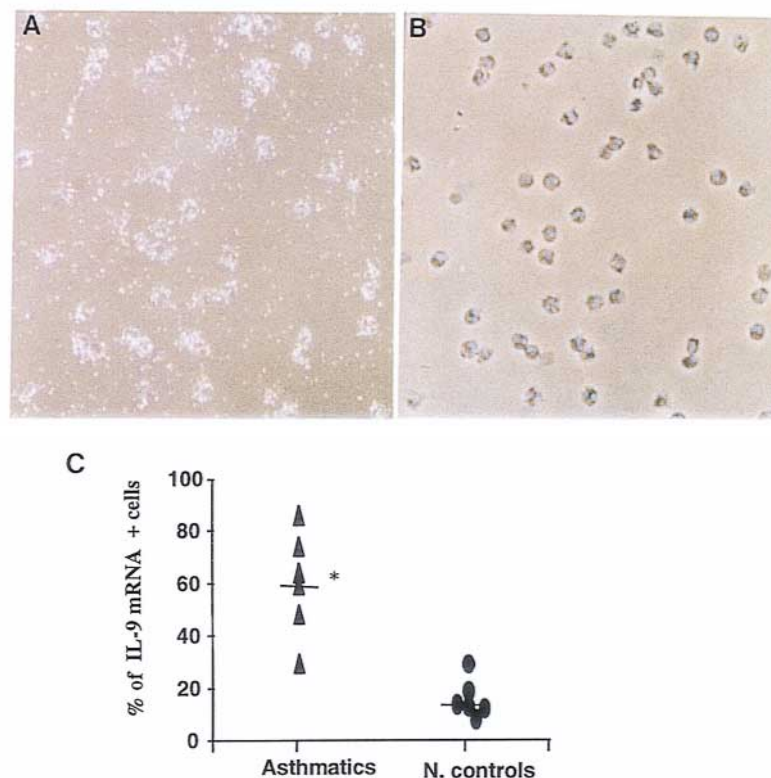


FIG 2. In situ localization of IL-9 mRNA expression in peripheral blood eosinophils. Representative examples of in situ hybridization of IL-9 mRNA using ^{35}S -labeled complementary RNA probes in human peripheral blood eosinophils are shown. **A**, In situ hybridization of IL-9 mRNA in eosinophils from an asthmatic subject (dark field, magnification 200 $\times$). **B**, Positive cells show eosinophil morphology with phase-contrast microscopy (magnification 200 $\times$). No specific signal could be detected with the sense-labeled riboprobe. **C**, IL-9 mRNA expression in nonatopic control and atopic asthmatic subjects. Results are expressed as the percentage of positive cells. * $P < .01$ for comparison between asthmatic and normal control subjects.

nificantly increased in asthmatic subjects ($n = 6$) compared with normal control subjects ($n = 6$; $56\% \pm 18\%$ vs $13\% \pm 7\%$, $P = .0049$; Fig 2, C).

Detection of IL-9 protein in human peripheral blood eosinophils

To further investigate the protein expression of IL-9 by human eosinophils, immunocytochemistry was performed by using IL-9-specific polyclonal antibody. A specific staining with anti-IL-9 was observed in peripheral blood eosinophils (Fig 3, A and C), with a higher level found in asthmatic patients ($n = 8$) compared with normal control subjects ($n = 10$; $71\% \pm 27\%$ vs $19\% \pm 30\%$, $P = .0063$; Fig 3, E). Substitution of the first antibody with goat Ig eliminated the immunostaining of the positive cells, demonstrating the specificity of the analysis (Fig 3, B and D). This result indicates that IL-9 protein expression is under microenvironmental control.

Effect of IL-1 β and TNF- α on human eosinophil release of IL-9 protein

TS1 survival and proliferation depends on the presence of murine IL-9 and are not supported by human cytokines.¹⁷ Ectopic expression of the human receptor

renders these cells responsive to the human ligand. RA3 cells form a stable TS1 cell line that express the human IL-9 receptor, which allows for human IL-9 maintenance. To further determine the biologic activity of IL-9 released by eosinophils and the factors involved in its regulation, we investigated whether RA3 transfectants proliferate on culture with eosinophil supernatants. RA3 transfectants were treated with either human IL-9 used as a positive control or serial dilutions of supernatants from eosinophils cultured for 18 hours either with IL-1 β , TNF- α , or medium alone. As shown in Fig 4, supernatants of human eosinophils from asthmatic patients induced a proliferation signal on RA3 transfectants, which is similar to that obtained with 30 pg/mL recombinant human IL-9 ($n = 3$). An increase of IL-9 release was detected in supernatants from IL-1 β - and TNF- α -stimulated eosinophils, compared with unstimulated eosinophils (>50%). No synergistic effect could be observed when eosinophils were stimulated with both IL-1 β and TNF- α (data not shown). Interestingly, eosinophil supernatants had no effect on proliferation of the nontransfected RA3 cell line TS1 (data not shown). These data indicate that peripheral blood eosinophils from asthmatic patients release IL-9 when cultured in

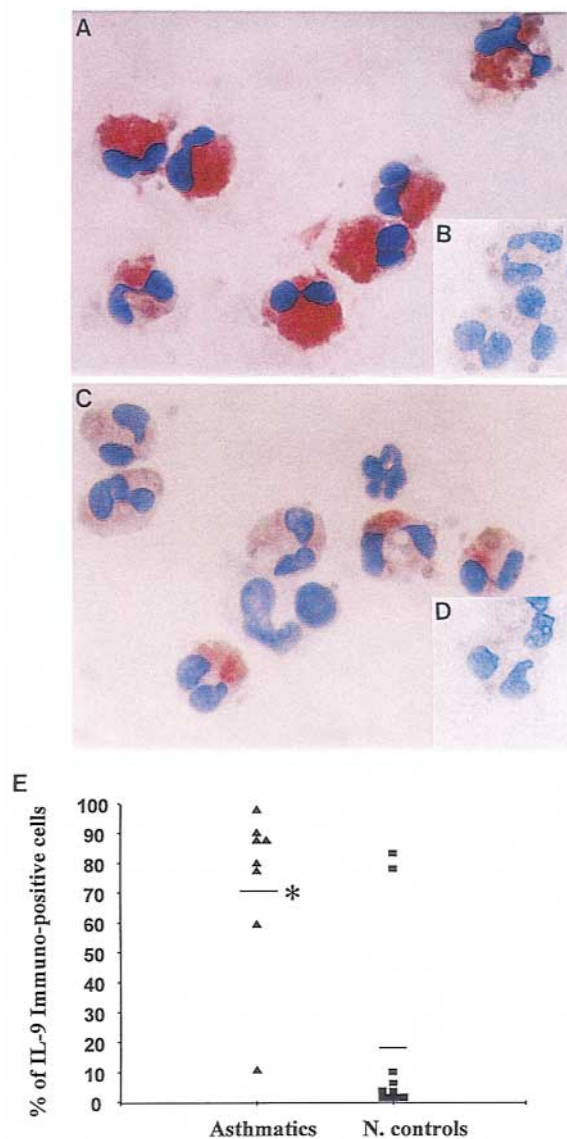


FIG 3. Detection of IL-9 protein in human blood eosinophils. Purified eosinophils from asthmatic subjects (**A**) and nonasthmatic, control subjects (**C**) were stained with goat polyclonal anti-IL-9 antibody. Goat IgG was used as a negative control (**B** and **D**). **E**, Percentage of IL-9 immunoreactive eosinophils from asthmatic and normal control subjects. Comparison between these two groups was determined by counting 500 cells per slide (* $P < .01$). The staining was performed by using goat anti-IL-9 followed by biotin-labeled, rabbit anti-goat streptavidin alkaline phosphatase, and counterstaining was with hematoxylin.

vitro and that the proinflammatory cytokines IL- β and TNF- α can induce this release.

DISCUSSION

Until recently, eosinophils were considered to be effector cells in asthma, without the capacity to generate cytokines. Evidence now exists to show that eosinophils produce a range of cytokines, including IL-3, IL-5, and

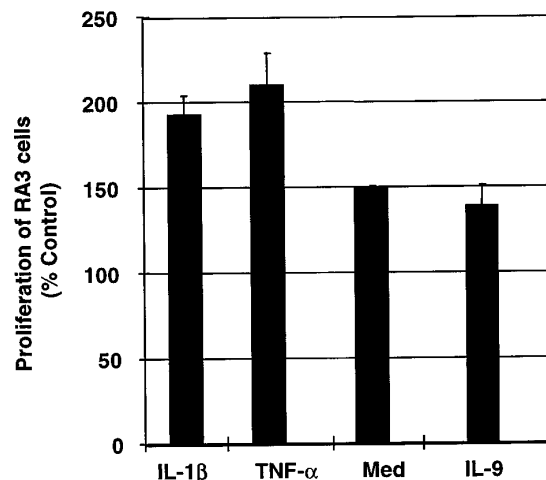


FIG 4. IL-9 release by human peripheral blood eosinophils. Human peripheral blood eosinophils were stimulated for 18 hours with recombinant IL-1 β , TNF- α , or both at 20 ng/mL or medium alone. IL-9 release was performed by biologic assay with RA3 cell proliferation, as described in the "Methods" sections. The RA3 cell line was stimulated with serial dilution of recombinant human IL-9 or medium alone (positive and negative controls, respectively). The y axis represents the ratio between the proliferation of RA3 cell line incubated with eosinophil supernatants under different conditions or IL-9 (30 pg/mL) and their proliferation when incubated with medium alone (control). Assays were performed in triplicate from the same donor. The data corresponds to the mean $\pm$ SD and are representative of 3 independent experiments performed under the same conditions.

GM-CSF.¹⁸⁻¹⁹ This report presents several lines of evidence demonstrating that human eosinophils can also express the potent T-cell mast growth factor IL-9. First, we detected IL-9 mRNA by using RT-PCR in eosinophils from both asthmatic and normal control subjects, as well as from the eosinophil-differentiated HL-60 cell line. To confirm this, we performed in situ hybridization, which allowed us to quantify the percentage of eosinophils expressing IL-9 message. Using immunocytochemistry, we confirmed that eosinophils can produce the IL-9 protein. Finally, we show by bioassay that IL-9 protein release from eosinophils is upregulated by TNF- α and IL-1 β .

IL-9 is a T_H2-type cytokine first described in the mouse as a T-cell and mast-cell growth factor.⁵⁻⁶ Recently, a role of IL-9 in asthma and allergy has been supported by the findings that it has pleiotropic activities on cell types associated with allergic diseases, including T_H2-type lymphocytes, mast cells, B cells, eosinophils, and airway epithelial cells.^{10,11} In particular, the biologic functions of IL-9 include its ability to promote proliferation and differentiation of mast cells and to enhance production of IgE in B cells.⁶⁻¹⁰

Recently, two independent studies with IL-9 transgenic mice showed that in vivo expression of IL-9 resulted in increased airways hyperresponsiveness.^{8,9} Similar to human asthma, these asthma models showed mucous hypersecretion, basement membrane thickening, increased intraepithelial mast cell numbers, lung eosinophilia, ele-

vated IgE levels, airways hyperreactivity, and increased responsiveness to antigen stimulation.^{8,9} Furthermore, several studies have demonstrated a strong correlation between the degree of inflammation, in particular eosinophilia, and airways hyperreactivity in asthmatic patients.²⁰ Our results provide new evidence suggesting that human eosinophils, through the expression and release of IL-9, may participate in modulating airways hyperreactivity.

The expression of IL-9 on human eosinophils was heterogeneous according to individual donors, and there was a significant difference between the percentage of IL-9⁺ eosinophils in asthmatic subjects compared with that found in normal control subjects, as determined by immunocytochemistry and in situ hybridization ($P < .01$). This is in line with our recent report demonstrating a high level of IL-9 expression in bronchial biopsy specimens from asthmatic subjects compared with normal control subjects and patients with sarcoidosis.²¹

An important new finding from our studies is the observation that IL-9 expression and release is upregulated by TNF- α and IL-1 β . TNF- α is a proinflammatory cytokine that has been shown to coordinate cellular responses, together with IL-1 β , that in turn orchestrates local inflammation. This local proinflammatory action is most likely mediated by activated inflammatory cells, inducing endothelial cells to express leukocyte adhesion molecules and inciting production of secondary cytokines and other inflammatory mediators.³ Recent evidence suggests that the production of TNF- α and IL- β is increased in asthmatic airways.²² Significantly elevated levels of TNF- α derived from cultured bronchoalveolar lavage cells have been reported in both stable and allergen challenged states.^{23,24} One can speculate that the mechanism underlying the effect of TNF- α and IL-1 β in airways inflammation may be mediated in part by activating eosinophils to produce and release IL-9. Thus the increased release of IL-9 by eosinophils after TNF- α and IL-1 β stimulation is in agreement with the airway eosinophilia observed in the asthmatic state.¹⁻³ Furthermore, in contrast to eosinophils from asthmatic subjects, spontaneous IL-9 release could not be detected in cultured eosinophils from normal control subjects, suggesting that eosinophils from asthmatic subjects may be activated in vivo. In accordance with that observation, several mediators have been shown to prime eosinophils and synergistically potentiate the activity of other stimuli for their degranulation and release of various factors, including cytokines.²⁵

The recruitment of leukocytes to inflammatory sites is mediated by the production of a number of chemoattractant mediators.³ IL-9 has also been reported to promote increased numbers of airway mast cells and eosinophils in IL-9 transgenic mice, as well as induce CC-type chemokines from airway epithelial cells.¹⁰⁻¹² Our results show that IL-9 could be expressed and released by human eosinophils. In the context of local allergic inflammation, the release of IL-9 by human eosinophils may be involved in the recruitment of inflammatory cells by enhancing the production of chemotactic factors.²⁶

In conclusion, the finding that human peripheral blood eosinophils express biologically active IL-9 suggests that the eosinophil may influence the recruitment and activation of effector cells linked to the pathogenesis of allergic disease.

We thank Ms Elsa Schotman for technical assistance.

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