

Eosinophil survival activity identified as interleukin-5 is associated with eosinophil recruitment and degranulation and lung injury twenty-four hours after segmental antigen lung challenge

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Background: Segmental antigen challenge in allergic volunteer subjects leads to the recruitment of inflammatory cells, including eosinophils, to the lung and to lung injury as shown by albumin influx into the alveolar air space. The goal of this study was to determine whether eosinophil-active cytokines, including IL-3, IL-5, or granulocyte-macrophage colony-stimulating factor, are released into the lung 24 hours after segmental antigen challenge of ragweed allergic subjects with allergic rhinitis and to determine whether the presence of the cytokine or cytokines is correlated with markers of lung inflammation and lung injury.

Methods: Volunteers underwent challenge with a wide variety of antigen doses, which resulted in the recruitment of inflammatory cell mixtures both with and without eosinophils.

Results: Eosinophil survival activity (ESA), the ability of the cytokine to prolong blood eosinophil survival in culture, was found in 5 of 17 ragweed allergic subjects and only in subjects challenged with relatively high doses of ragweed antigen (0.2 ragweed antigen units/ml or more). No ESA was found in bronchoalveolar lavage (BAL) fluid in any of eight nonragweed allergic subjects. This activity could be almost completely neutralized by preincubating BAL fluid with specific antibody to IL-5, although a small contribution by granulocyte-macrophage colony-stimulating factor may also have been present. ESA correlated with eosinophil recruitment ($r = 0.72$, $p < 0.001$) and degranulation in the lung ($r = 0.63$ to 0.81 , $p < 0.01$, for eosinophil granule constituents in BAL fluid) and lung injury as shown by albumin influx into the alveolar air spaces ($r = 0.83$, $p < 0.001$). ESA was unrelated to the presence of other inflammatory cells in the lung. Subjects who had IL-5 in BAL fluid appeared to undergo more severe initial reactions to antigen challenge.

Conclusions: We conclude that IL-5 is the most important constituent in ESA in the lung 24 hours after antigen challenge and that it correlates with eosinophil recruitment, degranulation, and lung injury. (*J ALLERGY CLIN IMMUNOL* 1993;92:607-15.)

Key words: Eosinophils, eosinophil survival activity, IL-5, IgE-mediated pulmonary inflammation, segmental antigen challenge

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The allergen-induced late phase response is associated with persistent bronchial obstruction, a diminished response to bronchodilators, increased bronchial reactivity, and airway inflammation.¹⁻⁵ Eosinophils are one of the predominant

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Abbreviations used

BAL:	Bronchoalveolar lavage
DCS:	Heat-inactivated defined calf serum
ESA:	Eosinophil survival activity
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL:	Interleukin
MBP:	Major basic protein
PIPES:	Piperazine- <i>N,N'</i> -bis-2-ethanesulfonic acid
RWAU:	Ragweed antigen unit (1 RWAU = 0.005 μ g/ml antigen E [Amb aI])

cell types found in bronchoalveolar lavage (BAL) fluid during the late phase response, and increased quantities of eosinophil granule-associated proteins are also present.^{6, 7} However, the mechanisms responsible for eosinophil recruitment and activation in the lung after antigen challenge are not known.

Recent work from a number of laboratories has demonstrated that cytokines, particularly granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, and IL-5, have the potential to play a major role in modulating eosinophil maturation, survival, and function.⁸⁻¹⁹ These three cytokines can all prolong eosinophil viability *in vitro*, cause eosinophils to become hypodense (a state associated with eosinophil activation), increase antibody-dependent cytotoxicity of eosinophils, and stimulate eosinophil leukotriene generation.⁸⁻¹⁹ Therefore these cytokines may play a critical role in the migration and activation of eosinophils in the airways *in vivo*. This hypothesis is supported by the observations that the gene cluster for IL-3, IL-5, and GM-CSF is upregulated in bronchoalveolar T lymphocytes in patients with atopic asthma and that levels of messenger ribonucleic acid for IL-5 are increased in bronchial biopsy samples from patients with asthma.²⁰⁻²² In contrast, patients with nonatopic asthma have a different pattern of T cell activation and cytokine production.²³ In addition, increased levels of IL-5, GM-CSF, and/or IL-3 have been observed in BAL fluid obtained 48 hours after local antigen challenge (references 21 and 24 and unpublished data of Ohnishi et al.). The goals of this work were to identify the most important eosinophil survival activity (ESA) 24 hours after local antigen challenge, to determine whether the presence of ESA is related to the dose of antigen used for local challenge, and to correlate the presence of ESA

with the presence of specific cells types in BAL fluid and with lung injury as shown by the influx of albumin into the airways.

In this series of experiments we have identified the most important ESA present in BAL fluid 24 hours after segmental antigen challenge in allergic volunteers as IL-5. In addition, with use of a wide range of allergen concentrations for challenge, which resulted in the recruitment of large numbers of inflammatory cells with and without the presence of eosinophils, the presence of ESA was associated with eosinophil recruitment and degranulation and with lung injury as shown by an influx of albumin into the alveolar air space. This work has been presented in preliminary form.²⁵

MATERIAL AND METHODS

Subjects

These procedures were approved by the Jefferson Medical College Institutional Review Board, and all subjects gave informed, written consent. Details of subject selection, bronchoscopy, antigen challenge, BAL, and cell handling were described previously.²⁶ Briefly, control, nonragweed allergic volunteer subjects had no history of allergic rhinitis and had a negative result on intradermal skin test with 100 ragweed antigen units (RWAU)/ml of crude ragweed antigen (1 RWAU = 0.005 μ g/ml antigen E [Amb aI] [lot L56-106, Greer Laboratories, Lenoir, N.C.]). Subjects allergic to ragweed had a history of fall allergic rhinitis and had a 2+ skin test reaction (greater than 8 to 10 mm wheal) 20 minutes after intradermal injection (0.02 ml) of 10 RWAU/ml or less of ragweed. The reconstituted ragweed solution was determined to be free less than 0.00125 endotoxin units/RWAU, E-Toxate assay, Sigma Chemical Co., St. Louis, Mo.) from contamination by endotoxin. All subjects were free of asthma, had normal pulmonary function, were taking no medication, had no serious medical problems, and were nonsmokers.

Segmental lung challenge and BAL

On the first study day, subjects underwent bronchoscopy after induction of anesthesia with topical lidocaine. The right middle lobe or lingula (randomized) was subjected to lavage (3 aliquots of 50 ml normal saline with syringe aspiration of fluid; BAL-1), followed immediately by local antigen challenge of the analogous, contralateral lung segment. Twenty-four hours later, the antigen-challenged segment was also subjected to lavage (BAL-2). A total of 25 2-day procedures was performed, eight in nonragweed allergic control subjects and 17 in ragweed allergic subjects.

Lavage fluid and cell processing

Fluid and cells from BAL were filtered through two layers of gauze and placed on ice. Total cell counts

were performed on uncentrifuged cells stained with Turk's solution with a hemocytometer, and differential cell counts were performed on cytocentrifuged preparations stained with Accustain (Sigma). Cells were then formed into pellets and lavage fluid was frozen in aliquots at -85°C .

Mediator measurements

Eosinophil purification. Eosinophils were isolated from peripheral blood as previously described.²⁷ Briefly, heparinized (10 U/ml) venous blood obtained from normal volunteers or patients with mild asthma or hayfever was sedimented with 6% dextran in 0.9% NaCl (Gentran 70, Travenol Laboratories, Deerfield, Ill.) at 5:1 (vol/vol) ratio for 45 minutes at 37°C . The buffy coat was collected and washed twice in PIPES buffer (25 mmol/L PIPES [piperazine-*N,N'*-bis-2-ethanesulfonic acid], 110 mmol/L NaCl, 5 mmol/L KCl, 25 mmol/L NaOH, 5.4 mmol/L glucose, pH 7.4; Sigma). The cells were suspended in 2.4 ml of Percoll (Sigma), density 1.070 gm/ml, with 5% heat-inactivated defined calf serum (DCS; Hyclone Laboratories, Logan, Utah) and overlaid on a discontinuous Percoll gradient of densities of 1.080, 1.085, 1.090, 1.100, and 1.120 gm/ml. Percoll osmolarities ranged from 290 to 315 mOsm/kg, and the pH was 7.4. Cells were centrifuged through the gradient at 1500 g in a model JA-20 fixed-angle rotor on a Beckman model J2-21 centrifuge (Beckman Instruments—Spinco Div., Palo Alto, Calif.) at 4°C for 45 minutes. Cell fractions were collected and eosinophil numbers were determined with the use of Randolph's stain. Eosinophil-rich fractions were pooled, washed twice with PIPES buffer with 1% DCS, and used immediately. The eosinophil preparations were greater than 80% pure and greater than 98% viable, as determined by staining with Randolph's stain and by trypan blue exclusion, respectively. Contaminating cells were neutrophils, and there was no contamination by lymphocytes or monocytes.

ESA. Recombinant human IL-5 was a generous gift from Schering-Plough Research (Bloomfield, N.J.), and recombinant human IL-3 and GM-CSF were purchased from Genzyme (Cambridge, Mass.). Rat monoclonal antibodies specific for IL-3, IL-5, or GM-CSF, specific for the respective cytokine with no cross-reactivity with other cytokines (Gleich GJ, Abrams JS. Unpublished observation.), were obtained from Schering. Preliminary studies showed that 10 μg of anti-IL-5, anti-IL-3, and anti-GM-CSF could completely neutralize 400 pg of IL-5, 100 pg of IL-3, and 8 pg of GM-CSF, respectively (data not shown). ESA was measured as a biologic activity that promoted the survival of eosinophils in culture *in vitro*.^{8, 12} Purified eosinophils were washed in Hybri-Care medium (American Type Culture Collection, Rockville, Md.) containing gentamicin (50 $\mu\text{g}/\text{ml}$) and 0.1% human serum albumin and resuspended in this medium supplemented with 10% DCS at 0.25×10^6 cells/ml. The cell suspension, 100 μl , was incubated with 100 μl of medium containing BAL fluid

(25% final concentration) or with control media for 4 days at 37°C in 5% CO_2 in 96-well flat-bottomed tissue culture plates. To determine which cytokine was responsible for ESA in these experiments, BAL supernatants with ESA were preincubated for 1 hour at 4°C with specific antibodies to IL-3, IL-5, or GM-CSF before BAL fluid was placed in the eosinophil culture system.²⁸ After 4 days of incubation, 120 μl of supernatant was removed from each well and 10 μl of fluorescein diacetate (0.2 mg/ml in phosphate-buffered saline) was added to the cell suspension.²⁹ After 30 minutes at 4°C , 10 μl of propidium bromide (2 mg/ml in Hybri-Care medium) was added to the cell suspension.³⁰ After an additional 5 minutes at 4°C , the numbers of viable cells (green fluorescence) and dead cells (red fluorescence) were counted with a hemocytometer and an epifluorescent microscope. Eosinophil viability was expressed as the percent of total cells still alive.

Albumin. Albumin was quantitated by a specific, competitive ELISA basically as described by Raphael et al.³¹

Eosinophil constituents. Major basic protein (MBP) was measured by an immunoradiometric assay with two monoclonal antibodies (J13-6B6 and J14-8A2; Wagner JM, Bartemes KR, Gleich GJ. Manuscript in preparation.), with a reference serum as a standard. A unit of MBP immunoreactive material in the serum standard was approximately equal to 1 ng of MBP; for simplicity, MBP is expressed in terms of nanograms per milliliter. Eosinophil-derived neurotoxin, eosinophil cationic protein, and eosinophil peroxidase were measured by double-antibody competitive radioimmunoassays as previously described.^{24, 32}

Neutrophil elastase. Neutrophil elastase was measured by an immunoradiometric assay developed with the use of an approach similar to that for MBP (Gleich GJ. Unpublished observation.). Nineteen mouse monoclonal antielastase antibodies generated from mice injected with neutrophil elastase (Elastin Products, Owensville, Mo.) were screened for their ability to bind ¹²⁵I-elastase (98% pure, Biodesign, Kennebunkport, Maine). The five antibodies that bound the highest quantities of elastase were screened in various combinations to determine the best pair (No. 1: 160-13E7-2E10-1012 and No. 2: 159-12G1-2E2-2C7) on the basis of the maximum quantity (counts per minute) of the second antibody bound, slope and range of titration curves, and degree of nonspecific binding. Immunol 4 wells (Dynatech Labs, Chantilly, Va.) were coated with affinity purified rabbit antimouse IgG capture antibody (lot No. 30438, Cappel Labs, West Chester, Pa.) used at a dilution of 1:100 in phosphate-buffered saline for 3 hours. After washing, the wells were coated again with protein A purified mouse monoclonal antielastase antibody No. 1 at a concentration of 5 $\mu\text{g}/\text{ml}$ overnight at 4°C . Dilutions (100 μl) of test sample or neutrophil elastase standard were incubated for 2 hours at 22°C , washed, and blocked with 1% normal mouse serum

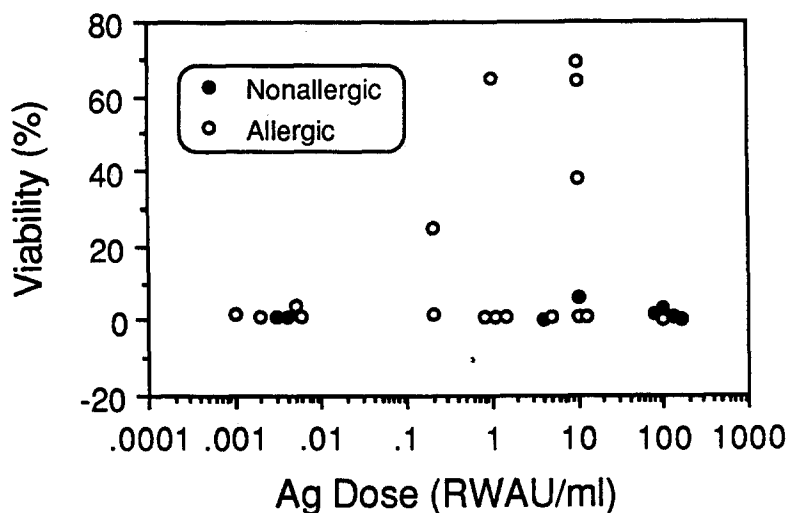


FIG. 1. ESA (eosinophil viability) as a function of the dose of antigen (Ag) used for segmental challenge. Plotted is highest concentration of ragweed antigen used for challenge in RWAU/ml (1 RWAU = 0.005 μ g of ragweed antigen E); 5 ml of antigen solution was used for challenge. Because several different subjects received same antigen concentrations of 0.005, 1, 10, and 100 RWAU/ml, individual data points have been moved laterally so that each individual data point can be observed. Data points represent mean of duplicate determinations. Correlation coefficient on logarithmically transformed data was 0.32 (p = not significant).

diluted in PPF-E buffer (0.1 mol/L sodium phosphate buffer with 1% protamine sulfate, 1% fetal calf serum, 0.01 mol/L ethylenediaminetetraacetic acid, and 0.1% sodium azide—pH 7.5) for 1 hour at 22° C. The wells were then washed and incubated with 50 ng/ml of ^{125} I-labeled antibody No. 2 for 1 hour at 22° C, washed, and counted in a Riasstar multichannel gamma counter (Packard Instruments Co., Meriden, Conn.) as described for the MBP assay. All assays were run with internal controls, and the interassay variability was less than 15%. The lower limit of detection of the assay was 0.25 ng/ml.

Statistical analysis

Statistical analyses were performed on Macintosh personal computers (Apple Computer, Inc., Cupertino, Calif.) with the use of StatView SE + Graphics (version 1.03, Abacus Concepts, Berkeley, Calif.) and the Primer of Biostatistics: The Program (version 1.0).³³ Data are presented as mean plus or minus SEM or as mean plus or minus the range (for assays performed in duplicate). Comparisons between ESA-negative and ESA-positive subjects were made by unpaired t tests. Regression analyses were performed with Cricket Graph (Cricket Software, Malvern, Pa.). A p < 0.05 was considered significant to reject the null hypothesis.

RESULTS

Three different cytokines, GM-CSF, IL-3, and IL-5, have been shown to enhance eosinophil survival in vitro.^{8, 10, 12, 15-17} Earlier work in our laboratory has demonstrated that the values for the dose of cytokine required for 50% survival of

eosinophils in an ESA assay for recombinant human IL-3, recombinant human IL-5, and recombinant human GM-CSF are 10, 10, and 1 pg/ml, respectively (unpublished data of Ohnishi et al.). Maximal eosinophil viability after 4 days in culture averages approximately 80% and viability is linearly related (on a semilog plot) to cytokine concentration (0.8 to 100 pg/ml for IL-3 and IL-5 and 0.16 to 10 pg/ml for GM-CSF; data not shown). When combinations of cytokines have been used in culture, no synergism has been noted. None of the eight nonallergic subjects who underwent challenge with ragweed antigen displayed significant ESA in BAL fluid obtained 24 hours after antigen challenge (BAL-2); eosinophil viability averaged $1.8\% \pm 0.7\%$ (range 0% to 6%). In procedures performed in allergic subjects, significant ESA was observed in 5 of 17 procedures, and in these 5 procedures eosinophil viability averaged $52.2\% \pm 8.7\%$ (range 25% to 69%). In the other 12 allergic subjects ESA averaged $1.3\% \pm 0.3\%$ (range 0% to 4%). As shown in Fig. 1, there was no direct relationship between dose of antigen used for segmental challenge and quantity of ESA ($r = 0.32$, p = not significant). There appeared to have been a minimum amount of antigen needed to trigger ESA production because subjects who underwent challenge with less than 0.2 RWAU/ml did not generate significant ESA. Antigen concentrations above that level led to production of ESA in 5 of 13 cases.

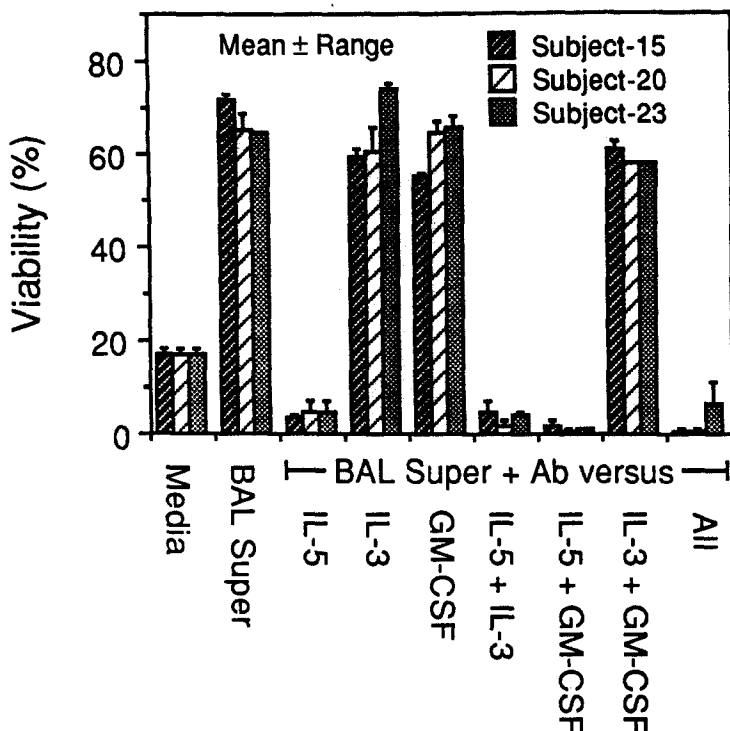


FIG. 2. Inhibition of ESA activity by antibodies (Ab) to IL-5. First two sets of data show ESA of media alone or supernatants (Super) from three most active BAL supernatants (subject 15, subject 20, and subject 23). Antibodies to cytokines indicated, either alone or in combination, were then added to these three BAL supernatants before their placement in ESA assay. Error bars indicate range of duplicate determinations.

To identify the ESA, BAL fluids with the greatest amount of ESA ($n = 3$) were incubated with antibodies to IL-5, IL-3, and GM-CFS, both individually and in combination, in the ESA assay. As shown in Fig. 2, ESA resided almost entirely in IL-5, although a minor contribution by GM-CSF cannot be ruled out. IL-3 appeared to contribute little to the ESA. These observations identify the ESA as primarily IL-5.

We next sought to determine whether significant differences could be found between ESA-negative subjects and ESA-positive subjects and whether the presence of IL-5 was correlated with other parameters associated with either lung inflammation or lung injury. For all subjects, all of the following parameters were comparable in the initial lavage fluid before antigen challenge (BAL-1): BAL fluid recovery ($53.2\% \pm 7.5\%$ to $57.4\% \pm 3.7\%$), total cells per milliliter ($152.9 \pm 17.0 \times 10^3/\text{ml}$ to $186.2 \pm 26.4 \times 10^3/\text{ml}$), and differential cell counts per milliliter (macrophages: $130.9 \pm 13.3 \times 10^3/\text{ml}$ to $158.4 \pm 24.8 \times 10^3/\text{ml}$; lymphocytes: $14.7 \pm 3.9 \times 10^3/\text{ml}$ to $18.8 \pm 4.1 \times 10^3/\text{ml}$; neutrophils $4.6 \pm 1.6 \times 10^3/\text{ml}$ to $8.4 \pm 1.2 \times 10^3/\text{ml}$; eosin-

ophils: $0 \pm 0 \times 10^3/\text{ml}$ to $0.6 \pm 0.4 \times 10^3/\text{ml}$). Therefore baseline values of lung cellularity did not differ between ESA-negative and ESA-positive subjects.

A direct comparison of BAL-2 cell composition, eosinophil and neutrophil granule products, and albumin concentration in ESA-negative and ESA-positive subjects is presented in Table I and Fig. 3. As can be seen in Table I, ESA-negative and ESA-positive subjects recruited approximately equivalent numbers of inflammatory cells to the lung, except for eosinophils. ESA-positive subjects recruited more than 10 times more eosinophils to the lung than ESA-negative subjects ($850.9 \pm 318.4 \times 10^3/\text{ml}$ versus $76.3 \pm 48 \times 10^3/\text{ml}$). Products of eosinophil degranulation, including MBP (37.82 ± 11.56 versus 7.78 ± 3.23 ng/ml), eosinophil-derived neurotoxin (107.1 ± 27.4 versus 15.1 ± 8.9 ng/ml), cationic protein (51.3 ± 18.3 versus 10.7 ± 4.9 ng/ml), and peroxidase (102.5 ± 23.9 versus 19.7 ± 11.0 ng/ml) were also significantly higher in ESA-positive subjects. Whereas both eosinophil number and products of eosinophil degranulation were higher in ESA-positive subjects, some overlap was observed with ESA-

TABLE I. Comparison of cellular and protein constituents of BAL-2 ub ESA-negative and ESA-positive subjects

Property	ESA negative (n = 12)	ESA positive (n = 5)	p Value*
Total cells/ml ($\times 10^3$)	1055.0 $\pm$ 337.6	1926.8 $\pm$ 747.6	0.23
Macrophages/ml ($\times 10^3$)	344.2 $\pm$ 108.5	336.9 $\pm$ 66.0	0.97
Lymphocytes/ml ($\times 10^3$)	58.0 $\pm$ 22.5	104.0 $\pm$ 61.5	0.39
Neutrophils/ml ($\times 10^3$)	576.5 $\pm$ 197.3	634.8 $\pm$ 455.8	0.89
Eosinophils/ml ($\times 10^3$)	76.3 $\pm$ 48.0	850.9 $\pm$ 318.4	0.002
Eosinophil products			
MBP (ng/ml)	7.78 $\pm$ 3.23	37.82 $\pm$ 11.56	0.004
Neurotoxin (ng/ml)	15.1 $\pm$ 8.9	107.1 $\pm$ 27.4	0.0008
Cationic protein (ng/ml)	10.7 $\pm$ 4.9	51.3 $\pm$ 18.3	0.009
Peroxidase (ng/ml)	19.7 $\pm$ 11.0	102.5 $\pm$ 23.9	0.002
Elastase (ng/ml)	383.9 $\pm$ 178.9	574.6 $\pm$ 273.3	0.57
Albumin (μ g/ml)	124.8 $\pm$ 54.6	691.4 $\pm$ 236.0	0.004

*Comparisons were performed by unpaired *t* tests.

negative subjects (Fig. 3). However, there was a strong positive correlation of ESA and eosinophil number and eosinophil granule constituents. The correlation coefficient between ESA and eosinophil number was 0.72 ($p < 0.001$), whereas the correlation coefficients between ESA and MBP, eosinophil-derived neurotoxin, eosinophil cationic protein, and eosinophil peroxidase were 0.81, 0.82, 0.63, and 0.77, respectively (all $p < 0.01$). There was no difference in neutrophil elastase concentration in ESA-positive versus ESA-negative subjects (574.6 ± 273.3 versus 383.9 ± 178.9 ng/ml; Table 1). Although ESA-positive subjects had a slightly higher skin test sensitivity to ragweed antigen than ESA-negative subjects (0.04 ± 2.51 versus 0.26 ± 2.19 RWAU/ml, geometric mean), this difference was not statistically significant ($p = 0.18$).

ESA-positive subjects also displayed significantly more lung injury, as reflected by albumin influx into the alveolar air space, than ESA-negative subjects. The albumin concentration in BAL fluid was more than fivefold higher in ESA-positive (691.4 ± 236.0 μ g/ml) than in ESA-negative (124.8 ± 54.6 μ g/ml) subjects (Table I). A strong correlation between ESA as measured by eosinophil viability and albumin influx into the airways ($r = 0.83$, $p < 0.001$) was also noted (Fig. 4).

DISCUSSION

Eosinophils appear to be important effector cells in the pathogenesis of bronchial asthma. A number of investigators have reported that the airways of patients with asthma contain an in-

creased number of eosinophils.^{7, 34, 35} Eosinophils synthesize and release leukotrienes, particularly leukotriene C₄, and release a variety of basic cytotoxic proteins.⁷ In addition, Djukanovic et al.³⁵ have recently reported that treatment of patients with asthma with an inhaled corticosteroid resulted in an increase in peak flow and FEV₁, a decrease in airway responsiveness to methacholine, and a decrease in asthma symptoms and β -agonist use, which was associated with a decrease in epithelial and mucosal mast cells and eosinophils.

Cytokines play a major role in modulating eosinophil maturation, viability, and function.^{8-19, 27, 28, 36} IL-3, IL-5, and GM-CSF induce differentiation and proliferation of eosinophil precursors, prolong the survival of peripheral blood eosinophils, and enhance their inflammatory properties including antibody-dependent cytotoxicity, superoxide generation, and degranulation.^{10, 12, 27, 36-40} Furthermore, IL-5 is a chemoattractant for eosinophils.⁴¹ Therefore these three cytokines are leading candidates as modulators of eosinophil function in the lung in vivo after antigen challenge.

The data presented in this manuscript have identified IL-5 as the cytokine that appears to be most important to ESA present in the lung 24 hours after segmental antigen challenge. A positive correlation was noted between ESA and the number of eosinophils recruited to the lung ($r = 0.72$, $p < 0.001$). It is possible that GM-CSF or IL-3, or both, also play an important role in this regard, perhaps in a compartment not accessible to BAL, or at a time point different from the

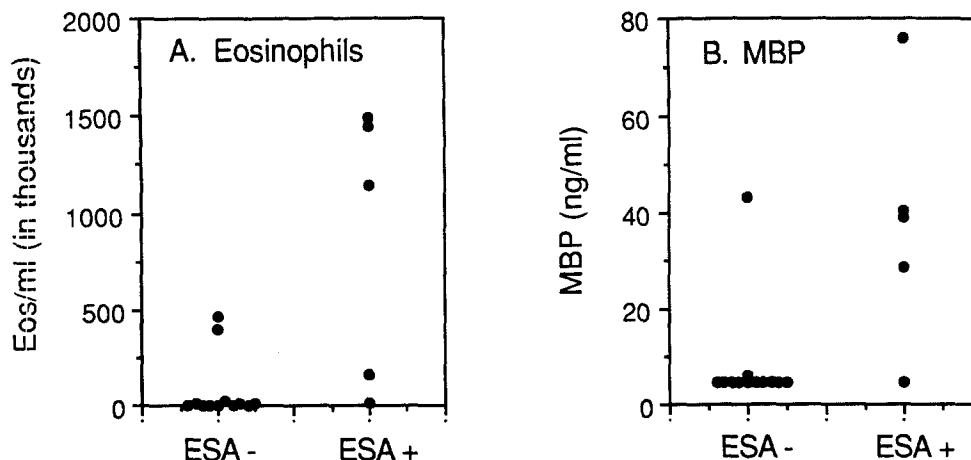


FIG. 3. Eosinophil (*Eos*) number (panel A) and MBP content (panel B) of ESA-negative and ESA-positive BAL supernatants. Limit of detection for MBP was 4.5 ng/ml.

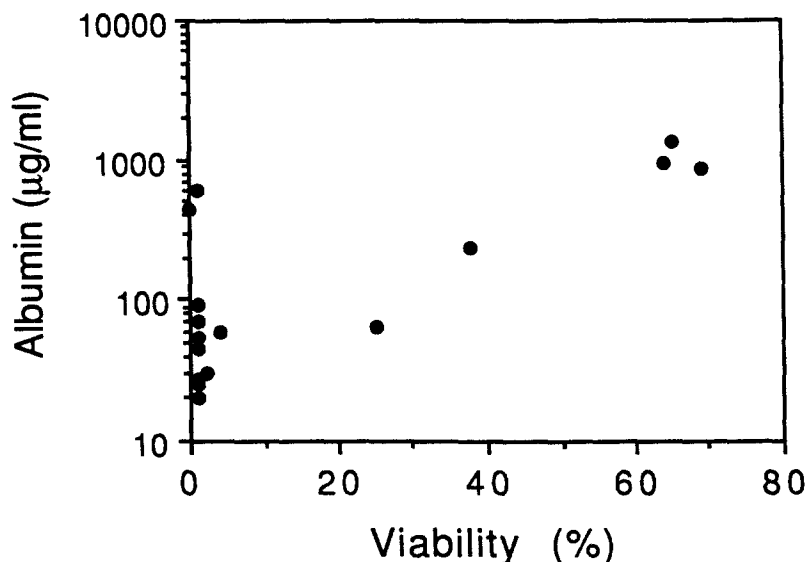


FIG. 4. Lung injury (BAL albumin concentration) as function of ESA activity (eosinophil viability). Regression analysis was performed on nontransformed (i.e., linear) data by Cricket Graph ($r = 0.83$, $p < 0.001$). For comparison, concentration of albumin in control BAL (BAL-1) for all 25 subjects in this series of experiments was 28.9 μg/ml.

one chosen for this study (24 hours after antigen administration). With use of the same bioassay, Ohnishi et al. (Unpublished data.) have also identified IL-5 as the most important component of ESA observed in BAL fluid 48 hours after antigen challenge. However, they have also observed the presence of GM-CSF in 8 of 11 subjects. Different potencies of IL-5 versus IL-3 and GM-CSF in the ESA assay do not appear to be able to explain the finding of IL-5 but not IL-3 or GM-CSF in this study. IL-5 and IL-3 are approximately equipotent in the ESA assay, whereas GM-CSF is approximately 10-fold more potent than either IL-5 or IL-3. Although a similar quantity of antigen was

used for challenge in this study and that of Ohnishi et al. (Unpublished data.), there were methodologic differences between the two studies. This study used a lyophilized preparation of short ragweed, whereas that of Ohnishi and his colleagues used an aqueous preparation of 50% short ragweed and 50% giant ragweed. Also, BAL was performed 24 hours after challenge in this study and 48 hours after challenge in the study by Ohnishi and his colleagues. A detailed kinetic analysis of the appearance of cytokines in BAL fluid after antigen challenge might shed light on this issue.

The events that lead to the production of ESA

and IL-5 are not clear. ESA-negative and ESA-positive subjects displayed similar skin test sensitivity to ragweed and recruited approximately equal quantities of other inflammatory cells to the lung. However, we have recently reported that antigen dose is an important factor that governs the quantity and type of inflammatory cells, particularly eosinophils, which are recruited to the lung 24 hours after antigen challenge.²⁶ We observed a strong correlation between antigen dose and eosinophils recruited to the lung ($r = 0.75$, $p < 0.001$). Although we observed a positive correlation between ESA and eosinophils recruited to the lung ($r = 0.72$, $p < 0.001$), there was no simple dose-response relationship between antigen dose and ESA. There may have been a minimum quantity of antigen that was required to induce the formation of IL-5, however, inasmuch as all of the subjects who produced measurable quantities of this cytokine underwent challenge with 0.2 RWAU/ml or more (Fig. 1). In addition, two of the five subjects in whom significant quantities of IL-5 were released had been subject to challenge both with a low and a high concentration of antigen. Measurable IL-5 was only observed after challenge with a high concentration of antigen.

Another factor that appeared to be related to the presence of IL-5 in BAL fluid 24 hours after local antigen challenge was the severity of the initial reaction produced by antigen challenge. We have previously reported that the control BAL procedure and the subsequent local antigen challenge resulted in a 10.6% decrease in FEV₁ and a 11.5% decrease in forced vital capacity (FVC) in spirometry measured immediately after this procedure on day 1.²⁶ In the five subjects in whom measurable IL-5 was released, FEV₁ decreased from 109.4% $\pm$ 4.6% of predicted to 91.2% $\pm$ 4.9% of predicted (a 16.6% decrease) and FVC decreased from 106.8% $\pm$ 4.5% of predicted to 88.6% $\pm$ 2.7% of predicted (a 17.1% decrease). These data suggest that the subjects who were subsequently found to have release of IL-5 into the airways had a more severe initial airway reaction. Similarly, in the two subjects who underwent challenge with both low and high concentrations of antigen, the low-concentration challenge resulted in a 7% decrease in FEV₁ and FVC whereas the high-dose challenge resulted in a 20% decrease in FEV₁ and FVC. Therefore an increased severity of the initial reaction also appears to be associated with the presence of IL-5 in the airways. Other key factors that determine whether ESA is generated in subjects after segmental challenge are not known.

The exact role played by IL-5 in the lung is not clear. In this study, IL-5, as shown by ESA, was associated with both eosinophil recruitment to the lung and with eosinophil degranulation, as shown by the presence of eosinophil granule products in BAL fluid. There was a notable association between ESA and eventual lung injury as shown by albumin influx into the airways. This suggests that IL-5 is an important mediator of eosinophil recruitment to the lung, eosinophil activation, and eventual lung injury. At the very least it is associated with these events and may be an important marker of these processes. Exactly how this process is controlled is not clear but is an area of interest. We will be able to more fully investigate the role of IL-5 in antigen-triggered lung inflammation when specific inhibitors of its synthesis or action are available for investigative use in human subjects.

Note added in proof. After this work was accepted for publication, the major ESA in BAL fluid 48 hours after antigen challenge was also reported to be IL-5 (Ohnishi, Kita, Weiler, Sur, Sedgwick, Calhoun, Busse, Abrams, Gleich. *Am Rev Respir Dis* 1993;147:901-7).

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