

Allergen challenge in asthma: association of eosinophils and lymphocytes with interleukin-5

Sur S, Gleich GJ, Offord KP, Swanson MC, Ohnishi T, Martin LB, Wagner JM, Weiler DA, Hunt LW. Allergen challenge in asthma: association of eosinophils and lymphocytes with interleukin-5. *Allergy* 1995; 50: 891–898. © Munksgaard 1995.

To test whether eosinophil recruitment after pulmonary allergen challenge is associated with interleukin (IL)-5 in patients with asthma, we performed segmental bronchoprovocation (SBP) with saline, and with low and high dosages of ragweed extract in six patients with allergic asthma. Bronchoalveolar lavage (BAL) of the challenged segments was performed 5 min after challenge (immediate BAL fluid) and repeated 24 h later (late BAL fluid). Allergen challenge resulted in recruitment of eosinophils, and increased levels of eosinophil-active cytokines. A bioassay showed the predominant eosinophil-active cytokine in the late BAL fluids to be IL-5. Analysis of the late BAL fluids revealed that IL-5 levels correlated with the numbers of eosinophils and lymphocytes. This study provides evidence that IL-5 is a critical cytokine associated with eosinophil and lymphocyte recruitment into the airways of patients with asthma following exposure to allergen.

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Key words: asthma; bronchoprovocation; eosinophils; interleukin-5; lymphocytes.

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Accepted for publication 5 July 1995

Considerable evidence suggests that asthma is an inflammatory disorder characterized by the presence of eosinophils in the airway submucosa, mucosa, and lumen (1–4). Eosinophils are also present in large numbers during the late airway response to segmental bronchoprovocation (SBP) with allergen (5, 6). Recruitment of eosinophils to the airways may involve eosinophil-active cytokines, such as interleukin (IL)-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are present in the BAL fluid of patients with asthma (7–9). The numbers of cells expressing IL-5 mRNA in the BAL cells and airway mucosa of patients with asthma are increased after allergen challenge (10, 11). Studies performed on patients with allergic rhinitis have shown that IL-5 is the predominant eosinophil-active cytokine detected during the late airway response to SBP with allergen (5, 12, 13). However, to our knowledge, the predominant eosinophil-active cytokine during the late airway response to allergen challenge has not been identified or quantified in patients with asthma.

In the present study, SBP with ragweed extract

was performed in six patients with allergic asthma. The results of our study indicate that IL-5 is the predominant eosinophil-active cytokine in the BAL fluid 24 h after allergen challenge. Moreover, the data support an association between IL-5 and recruitment of eosinophils and lymphocytes.

Material and methods

Study population

Six patients with mild asthma (14) and a history of exacerbation of allergic rhinitis and asthma symptoms during the ragweed pollen season were selected for this study (Table 1). All patients met the following inclusion criteria:

- 1) an immediate wheal-and-flare response of at least 5 × 5 mm to prick skin test with ragweed extract (Greer Co., Lenoir, NC, USA, Lot L56-106) at a 1:10 weight/volume dilution
- 2) a cutaneous late reaction as shown by a 10-mm diameter or greater induration 5–9 h after the prick skin test
- 3) an FEV₁ greater than 75% of predicted

Table 1. Patients' demographic data and dose of ragweed used for challenge

Patient no.	Age (years)	Sex	Wheal and flare (mm diameter)	Prick test ragweed late-phase cutaneous induration [†]	FEV ₁ * % predicted	SBP allergen dose, high (PNU) [§]
1	18	M	11×55	+	92	230
2	22	M	12×42	+	101	230
3	22	M	12×42	+	92	230
4	25	M	32×48	+	125	100
5	43	M	12×50	+	104	230
6	20	M	16×52	+	77	230

* FEV₁ refers to value just before saline or ragweed SBP.

[†] Induration > 10 mm diameter 5–9 h after prick skin test.

[§] Low dose was one-fourth of high dose.

- 4) no history of smoking for at least 2 years prior to SBP
- 5) absence of upper respiratory tract infection for at least 4 weeks prior to SBP
- 6) no need of either systemic or topical glucocorticoids or sodium cromolyn within 2 months of SBP.

All patients were asymptomatic at the time of SBP and gave written, informed consent. The study was approved by the Mayo Clinic Institutional Review Board.

Overall study design

All six patients underwent SBP with saline and a low dose and a high dose of ragweed in three lung segments (Fig. 1). SBP was performed with reconstituted lyophilized short ragweed extract (Greer Co., Lenoir, NC, USA, Lot L56-106). This same extract tested negative for endotoxin in an amebocyte lim-

ulus lysate assay (15). Endotoxin-free ragweed extract was selected for this study because we have shown that endotoxin contamination of the allergen causes neutrophilia (instead of eosinophilia) following SBP (15). The high dose of ragweed for endobronchial challenge was selected on the basis of the immediate prick skin test size by a modification of the method of Wenzel et al. (16). In five patients, the high-allergen dose was 230 protein nitrogen units (PNU) of ragweed extract. However, in patient 4, who had previously experienced severe seasonal asthma symptoms, 100 PNU was selected for the high dose (Table 1). A dose of one-fourth of the high-allergen dose was selected for the low-allergen dose. All allergens were suspended in 5 ml normal saline, and 5 ml normal saline was used for the sham challenge. Bronchoscopy with bronchoalveolar lavage (BAL) was performed at 5–8 min (immediate lavage) and again 24 h (late lavage) after challenge.

Bronchoscopy and bronchoalveolar lavage (BAL)

The overall plan of bronchoscopy and BAL is summarized in Fig. 1. All patients were admitted to the Clinical Research Study Unit at the Mayo Clinic for bronchoscopy. The procedure used an Olympus P20D bronchoscope via the oral route. The topical anesthetic agents used were benzocaine (20%) and lidocaine (2%). In addition, 0.5–2 mg of midazolam was given intravenously as needed to control anxiety and reduce cough. The bronchoscope was advanced to the subsegmental level until it could not be advanced any further, and from this “wedged” position, a 5-ml saline challenge was instilled (segment 1). The wedge was maintained for an additional 5–8 min, after which BAL was performed with 100 ml of 37°C normal saline. The immediate BAL fluid from segment 1 was termed “baseline”. In a nearby segment (segment 2), a low dose of allergen was administered, followed by BAL in 5–8 min. The bronchoscope was then moved to a segment in the opposite lung (segment 3) where the high dose of

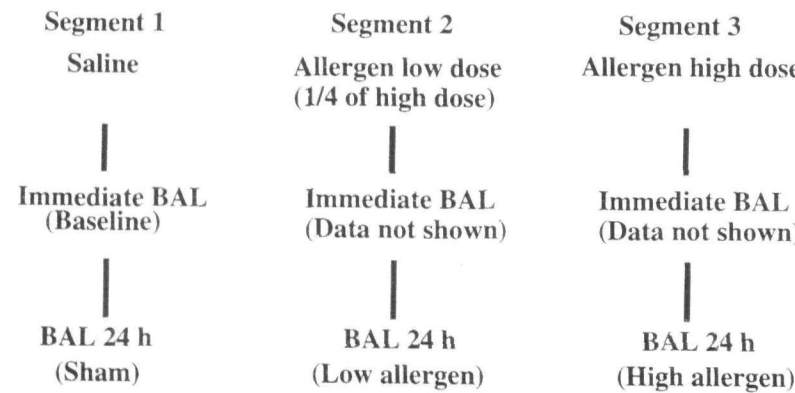


Fig. 1. Segmental bronchoprovocation. SBP was performed with saline (segment 1), low dose of ragweed extract (segment 2), and high dose of ragweed extract (segment 3). BAL was performed 5–8 min after challenge in segment 1 to yield “baseline” BAL fluid. BAL was also performed in segments 2 and 3 after 5–8 min. Because cell counts and assays performed on BAL fluids obtained 5–8 min after challenge in segments 2 and 3 were not statistically different from baseline (*P* values ranged from > 0.1 to 0.48), these data are not presented. BAL was repeated 24 h later in segments 1, 2, and 3 to yield sham (saline challenge), low-allergen (low-dose allergen challenge), and high-allergen (high-dose allergen challenge) BAL fluids.

allergen was similarly administered, followed by BAL in 5–8 min. The immediate BAL fluids from segments 2 and 3 were comparable to the baseline BAL fluids in cell numbers and were not analyzed further (data not shown). Bronchoscopy with BAL was repeated in the saline and the two allergen-challenged segments 24 h later. The BAL fluid obtained 24 h after saline challenge was identified as “sham”. Likewise, the BAL fluids 24 h after low-dose and high-dose allergen challenge were identified as “low allergen” and “high allergen”, respectively (Fig. 1).

Processing of BAL fluids and cells

The BAL fluids were passed through nylon mesh and centrifuged at 200 g for 10 min. After centrifugation, the BAL fluids were frozen at -20°C for later analyses. The pelleted BAL cells were resuspended, and cytocentrifuge preparations were stained with Diff Quick (Baxter Healthcare Corp., Miami, FL, USA). Differential cell counts were performed by enumerating macrophages, lymphocytes, neutrophils, and eosinophils in the stained slides. A minimum of 400 cells per preparation was counted. The total counts of each cell type were determined by multiplying the differential cell count by the total number of BAL cells from the segment.

In order to determine the extent of eosinophil and neutrophil degranulation in the challenged segments. BAL fluids were analyzed for eosinophil cationic protein (ECP) and for neutrophil elastase, respectively. In addition, eosinophil-active cytokines (such as IL-5, IL-3, and GM-CSF) were evaluated by a sensitive bioassay which detects these cytokines, the eosinophil survival assay (see below); antibody blocking assays (see below) were performed on the BAL fluids to determine the predominant cytokine present (12, 13). The IL-5 levels in BAL fluids were quantitated by an immunoradiometric assay (see below).

Immunoassay of ECP and neutrophil elastase

Double antibody-inhibition radioimmunoassays were used to measure ECP as previously described (17). Briefly, ECP in these test samples competes with ^{125}I -labeled ECP for binding to polyclonal rabbit anti-ECP. A two-site immunoradiometric assay was used to measure neutrophil elastase in the BAL fluids, as described previously (13).

Eosinophil survival assay

This test has been described elsewhere (12, 13, 18) and takes advantage of the ability of cytokines to prolong eosinophil survival to determine the pres-

ence and identity of cytokines. Briefly, Percoll gradient purified normodense eosinophils ($>80\%$ pure and 98% viable in Hybri-Care with 10% defined calf serum) were incubated at 37°C , 5% CO_2 , with $25\text{ }\mu\text{l}$ BAL fluid at a final concentration of 2.5×10^4 eosinophils per $200\text{ }\mu\text{l}$. The percentages of viable eosinophils after 4–5 days were determined using fluorescein diacetate (stains live cells green) and propidium iodide (stains dead cells red). The stained cells were visualized under UV light in a Zeiss standard photomicroscope (Carl Zeiss, Inc., Oberkochen, Germany). The eosinophil viability-enhancing activity (EVEA) was defined as percent viability of eosinophils incubated with BAL fluid minus the percent viability of eosinophils incubated with medium alone. Percent viability of eosinophils was the ratio of live eosinophils to live plus dead eosinophils.

The predominant cytokine responsible for the EVEA was determined by assays using monoclonal antibodies to IL-3, IL-5, or GM-CSF, as described elsewhere (12, 13, 18). Briefly, BAL fluids with high EVEA were incubated with medium (no antibody) or with monoclonal antibodies to IL-3, IL-5, or GM-CSF separately or in various combinations for 1 h at room temperature to block the bioactivity of the cytokines. The EVEA of these blocked samples was then determined, as outlined above.

IL-5 immunoradiometric assay

A two-site immunoradiometric assay was developed to measure IL-5, as previously described. The lower limit of detection of the assay was 13 pg/ml . The assay failed to detect recombinant human GM-CSF, IL-3, and IL-4, indicating that it was not cross-reactive with these cytokines. BAL samples with IL-5 levels below the detection limits of this assay were concentrated fivefold, using Centricon 10 concentrators (Amicon Inc., Beverly, MA, USA) and reanalyzed. The final results were expressed as pg/ml unconcentrated BAL fluids. To define the level of IL-5 in the BAL fluids of normal individuals, 11 BAL samples were obtained. The normal individuals met the following criteria:

- 1) age range 18–55 years
- 2) never a smoker
- 3) no history of asthma or allergic disease
- 4) prick skin test negative to a battery of eight common allergens including birch, ragweed, June grass, *Dermatophagoides pteronyssinus* and *D. farinae*, cat, *Alternaria*, and cockroach
- 5) no pulmonary or significant systemic disease
- 6) not receiving systemic or inhaled glucocorticoids for the past year.

For the purpose of the present study, elevated BAL IL-5 levels were defined as greater than the

mean value of the normal individuals plus two standard deviations.

Statistical analyses

While not the central aim of this study, we tested the effect of allergen dose on various BAL parameters measured. These analyses were complicated by the observation that eosinophils were recruited to the sham segment in two patients. Eosinophil recruitment to the sham segment has been reported by other investigators (5). It is therefore possible that sham may not be the best control to determine an allergen dose-effect. Therefore, the effects of allergen dose on various BAL parameters were determined using two linear regression approaches; first, taking baseline as zero allergen dose and, second, using sham as zero allergen dose. For the independent (x)-variable in both methods, a value of 1 was assigned to the low-allergen and a value of 4 to the high-allergen segments to reflect the relative dosing of low allergen which was one-fourth of high allergen. The responses to allergen challenge were graphed against allergen dose, and the slopes of the dose-response relationships were determined for each of the six patients. The mean of the individual slopes was tested against zero using a one-sample t -test. When baseline was taken as zero allergen dose, P values were determined by a two-tailed test. However, when sham was taken as zero allergen dose, both one-tailed and two-tailed tests were performed. The use of one-tailed test while taking sham as zero dose may be justified because only elevation of a BAL parameter in the low-allergen and high-allergen segments compared to sham was tested. To assess association between various BAL parameters, two statistical approaches were used. First, Pearson correlations were determined on log transformed values in the sham, low-allergen, and high-allergen segments. Second, the relationship between IL-5 and

eosinophils and lymphocyte numbers was also tested by performing a linear regression of one measure against another. Values from all six BAL segments (three immediate, 5–8 min after challenge, and three late, 24 h after challenge) were used to determine individual patient slopes; the mean slope from six patients was tested against zero.

Results

Recruitment of cells after SBP

The sham baseline BAL fluids contained predominantly (>80%) macrophages with little variation among patients. Likewise, the predominant (>80%) cell in the immediate low- and high-allergen BAL fluids was the macrophage (data not shown). Table 2 shows the eosinophil, neutrophil, lymphocyte, and macrophage numbers in BAL fluids. When baseline was taken as zero allergen dose, there was a significant effect of allergen on recruitment of eosinophils ($P < 0.01$) and neutrophils ($P < 0.05$). However, when sham was taken as zero allergen dose, eosinophil numbers ($P = 0.05$), but not neutrophil numbers, achieved significance with a one-tailed test. In four of six patients, the eosinophil numbers in the sham segment were less than 0.06×10^6 . However, in two patients, eosinophils were also recruited to the sham segment (patient 3, 21×10^6 ; patient 5, 2.5×10^6).

ECP and neutrophil elastase levels in BAL fluids after SBP

Table 2 shows the measurements of ECP and neutrophil elastase in BAL fluids. When baseline was taken as zero allergen dose, a significant dose-effect was observed with ECP ($P < 0.05$) and elastase ($P < 0.005$). However, these comparisons did not achieve significance when sham was taken as zero allergen dose.

Table 2. Effect of allergen dose on BAL parameters*

	Baseline	Sham	Low allergen	High allergen	P value [†]	P value [‡]
Eosinophils $\times 10^6$	0.03 (0.01)	3.94 (3.43)	8.16 (3.43)	19.8 (10.6)	<0.01	<0.05
Neutrophils $\times 10^6$	0.17 (0.05)	4.9 (3.8)	3.1 (1.1)	2.14 (0.61)	<0.05	—
Lymphocytes $\times 10^6$	0.36 (0.11)	1.78 (1.3)	2.2 (0.9)	2.1 (0.98)	NS	NS
Macrophages $\times 10^6$	7.58 (1.53)	8.38 (4.72)	8.3 (2.3)	4.4 (0.6)	NS	—
ECP ng/ml	1.0 (0)	11.0 (6.8)	11.5 (3.6)	33.2 (14.4)	<0.05	NS
Elastase ng/ml	3.7 (0.7)	204 (103)	110 (38.5)	161 (91)	<0.005	—
EVEA %	0.83 (0.3)	8.8 (7.4)	15.2 (3.6)	19.8 (9)	<0.02	<0.02
IL-5 pg/ml	4.9 (1)	160 (149)	155 (85)	1322 (539)	<0.02	<0.05

* Data are mean values (standard error of means).

[†] Two-tailed P values, taking baseline as zero allergen dose, and low allergen and high allergen as one and four allergen doses, respectively. Responses to allergen challenge were graphed against allergen dose, and slopes of dose-response relationships were determined for each of six patients. P values were determined by testing mean of individual slopes against zero by one-sample test.

[‡] One-tailed P values, taking sham as zero allergen dose, and low allergen and high allergen as one and four allergen doses, respectively. None of two-tailed P values were significant. When mean value of BAL parameter in high-allergen segment was less than sham, one-tailed test was not performed (—).

NS: Not significant.

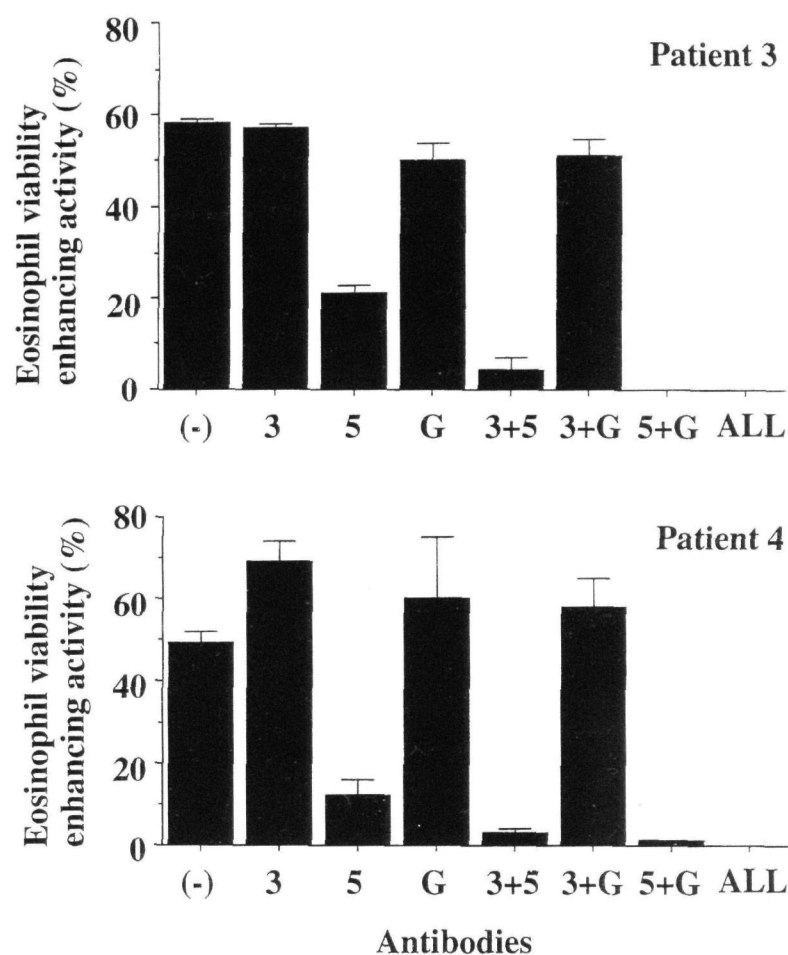


Fig. 2. Blocking of BAL fluid bioactivity in eosinophil survival assay by monoclonal antibodies to cytokines. BAL fluids from two patients with high eosinophil viability enhancing activities (EVEA) were analyzed using monoclonal rat anti-IL-3, anti-IL-5 (5), and anti-GM-CSF (G) antibodies. Most EVEA was inhibited by anti-IL-5, suggesting that predominant cytokine in BAL fluids is IL-5. When combinations of antibodies (anti-3 + 5, or 5 + G, or 3 + 5 + G) were used, cytokine activity was reduced below that of 5 alone, indicating that small amounts of IL-3 and GM-CSF were also present.

Eosinophil active cytokines in BAL fluids after SBP

Table 2 shows the EVEA of the BAL fluids. When baseline was taken as zero allergen dose, a significant dose-effect was observed with EVEA ($P < 0.02$). However, when sham was taken as zero allergen dose, EVEA achieved significance only with a one-tailed test ($P < 0.02$).

Fig. 2 shows the result of blocking assays performed on the late BAL fluids obtained from the allergen-challenged segments from patients 3 and 4, two patients with high EVEA (patient 3 = 58%; patient 4 = 49%). Analyses of the effect of monoclonal antibodies to cytokines revealed that anti-IL-5 was the only antibody which, by itself, had an impressive effect on survival activity; in contrast, anti-IL-3 or anti-GM-CSF had little effect on viability. Moreover, the combinations of anti-IL-3 and anti-GM-CSF had essentially no effect on survival activity, whereas inclusion of anti-IL-5 with either anti-IL-3 or anti-GM-CSF, or a combination of all three monoclonal antibodies, was even more effective than anti-IL-5 alone. The data from these two patients suggest that the predominant eosinophil-active cytokine in the BAL fluids 24 h after challenge was IL-5 and that small amounts of IL-3 and GM-CSF were also present. Similar results were obtained from patient 6 (data not shown).

IL-5 levels in the BAL fluids after SBP

Table 2 shows the IL-5 levels in BAL fluids. When baseline was taken as zero allergen dose, a significant dose-effect was observed with IL-5 ($P < 0.02$). However, when sham was taken as zero allergen

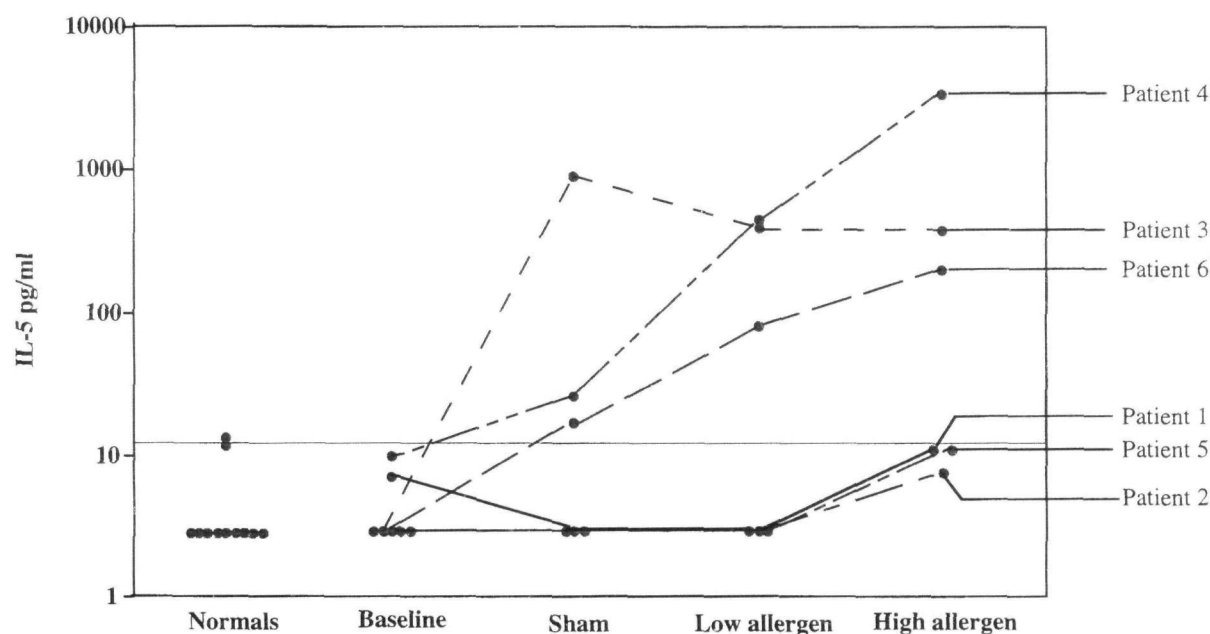


Fig. 3. IL-5 levels in BAL fluids from individual patients after SBP, as determined by immunoradiometric assay. All values are expressed as pg/ml unconcentrated BAL fluid. If IL-5 levels were below detection (assay limit of 3 pg/ml), value of 3 was assigned to them. For normal subjects (normals), each circle represents one subject. Baseline, sham, low allergen, and high allergen are as previously defined in Fig. 1. Vertical axis shows IL-5 levels on logarithmic scale.

dose, IL-5 levels demonstrated a significant dose-effect only with a one-tailed test ($P = 0.05$). Fig. 3 shows the IL-5 levels in baseline, sham, and low-allergen and high-allergen segments in individual patients and compares these levels to those detected in normal subjects.

Correlations among BAL parameters

Pearson correlations performed on the values of BAL parameters in the sham, low-allergen, and high-allergen segments revealed several associations. First, the eosinophil numbers significantly correlated with lymphocyte numbers (but not neutrophils or macrophages) in all three segments ($r > 0.86$, $P < 0.05$). Second, EVEA correlated with eosinophil ($r > 0.83$, $P < 0.05$) and lymphocyte numbers ($r > 0.93$, $P < 0.01$), as well as IL-5 levels ($r > 0.93$, $P < 0.01$). Finally, IL-5 levels correlated with eosinophil ($r > 0.92$, $P < 0.01$) and lymphocyte numbers ($r > 0.92$, $P < 0.01$). The relationship between IL-5 and eosinophil and lymphocyte numbers was also tested by performing a linear regression of one measure against another (Fig. 4). This method of analysis also revealed a significant association between IL-5 and eosinophils. A similar trend was seen between IL-5 and lymphocytes in five of six patients, but not in patient 1, who did not have an eosinophilic or lymphocytic response to allergen challenge. When the data were analyzed without this outlier (patient 1), the regression was significant for lymphocytes ($P < 0.01$). Taken together, the two methods of statistical analyses support an association between IL-5 and eosinophil and lymphocyte numbers.

Discussion

The overall goal of this study was to identify and quantify the predominant eosinophil-active cytokines in the BAL fluid and determine their association with the number of eosinophils recruited. We found that the EVEA in the late (24-h) BAL fluids correlated with the numbers of eosinophils and lymphocytes, suggesting that eosinophil-active cytokines are associated with eosinophil and lymphocyte recruitment. The predominant eosinophil-active cytokine in the BAL fluid in the ragweed-challenged segments was identified as IL-5. In previous studies, IL-5 was the predominant eosinophil-active cytokine in the BAL fluids from patients with allergic rhinitis after SBP with ragweed (12, 13). The present study extends these findings to allergic asthma.

In the present study, IL-5 levels in unconcentrated BAL fluids were not elevated in the baseline BAL fluids from the six patients with asymptomatic allergic asthma. This result contrasts with that of a pre-

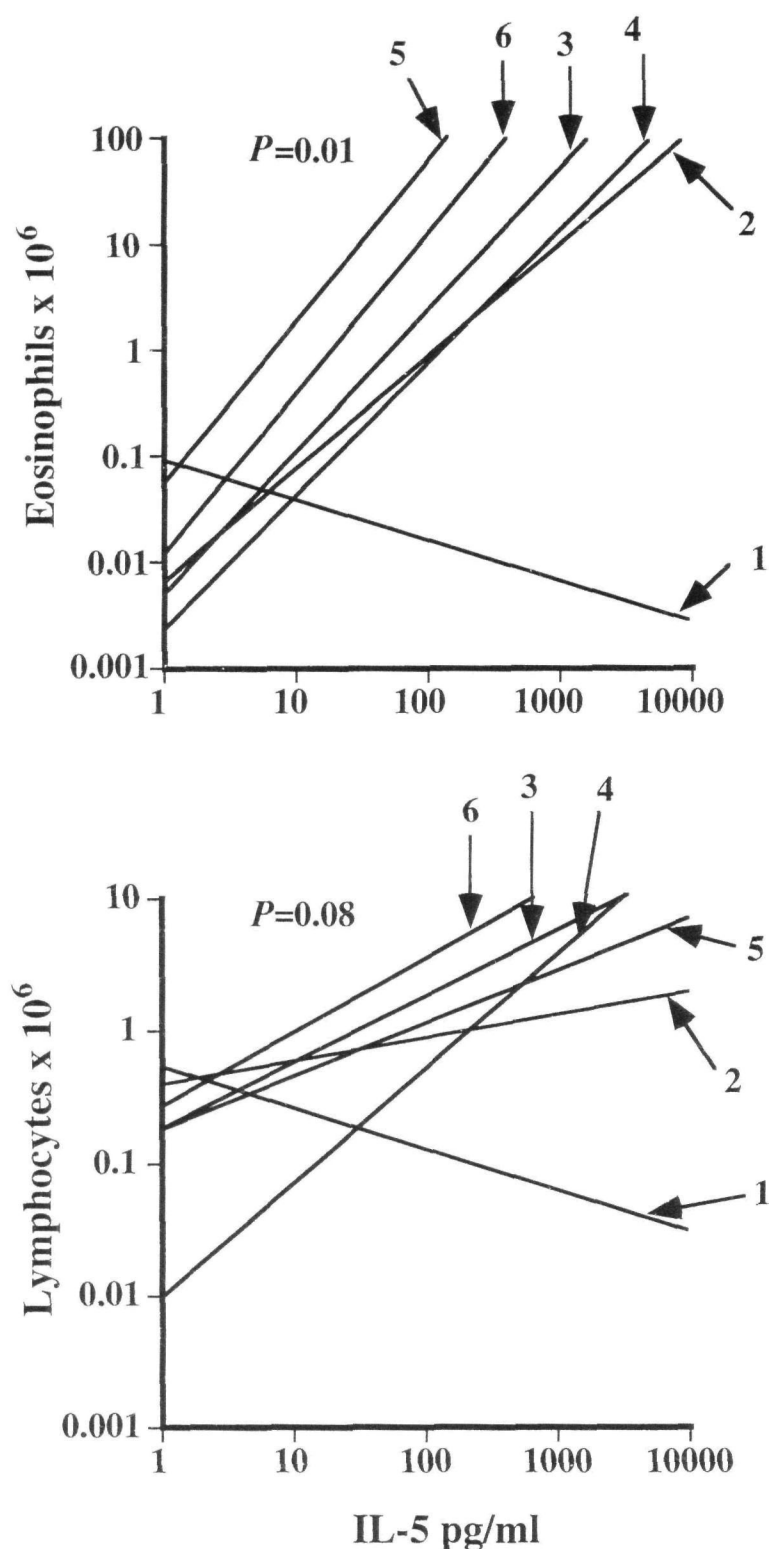


Fig. 4. Linear regression slopes between IL-5 and eosinophil and lymphocyte numbers. Data from all six BAL segments (three immediate, 5–8 min after challenge, and three late, 24 h after challenge) were used to determine individual patient slopes. Two-tailed P values are at top of each panel and were determined by testing mean slope against zero. Upper panel shows linear regression between IL-5 and eosinophil numbers, while lower panel shows linear regression between IL-5 and lymphocyte numbers. Numbers 1–6 indicate linear regression slopes of patients 1–6. Positive correlations are indicated by upward slope, as observed in patients 2–6 in both panels. Patient 1 had negative slope in both panels; eosinophils and lymphocytes were not recruited in this patient in low-allergen and high-allergen segments. When lower panel data were reanalyzed without this outlier (patient 1 data), regression was significant for lymphocytes.

vious study which found elevated IL-5 levels in BAL fluids from patients with asymptomatic allergic asthma (8). While 20-fold concentration of BAL fluid

and use of a bioassay may have allowed more sensitive detection of IL-5 in the study by Walker et al. (8), another explanation for this difference is that the patients in the present study had milder asthma and were completely asymptomatic at the time of study. Because Walker et al. used the IL-5-sensitive $L_yH_7B_{13}$ cell line to measure IL-5, it is also possible that this bioassay detected a cytokine other than IL-5 (neutralizing antibodies to IL-5 were not employed to confirm their results). Remarkably high IL-5 levels (3.3 ng/ml and 1.5 ng/ml) were found in two patients in the present study, and the total amounts of IL-5 produced in the challenge segments were 165 and 75 ng, respectively (IL-5 concentration/ml $\times$ volume of BAL fluid (ml)). As noted above, the high levels of IL-5 in the sham segment of patient 3 may have been due to antigenemia following SBP (5). In three patients (Fig. 3), BAL IL-5 levels in low- and high-allergen challenge segments were not elevated. Why these patients did not respond to ragweed challenge is not clear; the most likely explanation is the need for an increased concentration of allergen. Alternatively, this could have been due to the general difficulty and variability in measuring cytokines in BAL fluids, even after concentration.

The two methods of statistical analyses used in our study support an association between the IL-5 levels and the total numbers of eosinophils and lymphocytes in the 24-h BAL fluids in patients with allergic asthma. In patients with allergic rhinitis, Sedgwick et al. have demonstrated that BAL eosinophil numbers correlated with IL-5 levels after SBP with allergen (5), but they did not report an association between IL-5 and lymphocyte numbers. The association between IL-5 levels and the numbers of cells recruited may have several explanations. First, eosinophils and lymphocytes in the BAL may have produced IL-5, as suggested by previous studies (19, 20). Second, IL-5 could be produced by one cell type, for example, the lymphocyte (21) and, in turn, could recruit the other cell type, the eosinophil. Third, IL-5 could be produced by a third cell type, such as the mast cell in the airway mucosa, and could recruit both eosinophils and lymphocytes. The presence of $Fc_\epsilon RI$ on mast cells would allow binding of ragweed-specific IgE which, when cross-linked, could cause upregulation of IL-5 mRNA; for example, in mice, cross-linking of $Fc_\epsilon RI$ on mast cells has been shown to upregulate IL-5 mRNA (22). Evidence that mast cells also produce IL-5 protein comes from a study which showed that 14–60% of IL-5 immunoreactive cells in the nasal mucosa of patients with rhinitis were mast cells (23).

While this was not the central aim of this study, we tested the effect of allergen dose on the various BAL variables measured. These analyses were complicated by the finding, in two patients, of an unex-

pected recruitment of eosinophils in the sham segment. It is unlikely that this finding was due to carry-over of allergen in the bronchoscope from the allergen-challenged segment to the saline segment because the challenges were performed in the order of saline, low-dose allergen, and high-dose allergen. Eosinophil recruitment to the saline-challenged segment after SBP has been reported previously and may be due to generalized pulmonary response due to antigenemia (5). Alternatively, spillage of ragweed allergen from either the low-dose allergen or the high-dose allergen segments to the sham segment may have occurred from coughing during SBP or in the 24 h following SBP. Our results, taken together with this previous report of eosinophil recruitment to the sham segments, suggest that sham values may not be the best control for evaluating allergen dose-effect. To address this issue, the baseline segment was taken as zero allergen dose in addition to taking sham as zero allergen dose. These two methods of analyses, namely, taking baseline as zero allergen dose and sham as zero allergen dose, suggest that eosinophil numbers, EVEA, and IL-5 level in BAL fluid show a dose response to allergen. It is possible that the dose response would be more pronounced if higher concentrations of ragweed were used for SBP. However, for safety reasons, it is difficult to justify usage of high doses of allergens for SBP in patients with asthma, as opposed to patients with allergic rhinitis.

In summary, the results of the present study indicate that IL-5 is the predominant eosinophil-active cytokine in the BAL fluids 24 h after SBP with ragweed in patients with asthma. This study also supports an association between the levels of IL-5 and the numbers of eosinophils and lymphocytes recruited. We suggest that IL-5 is a critical cytokine associated with eosinophil and lymphocyte recruitment in patients with allergic asthma after allergen exposure.

Acknowledgments

We thank Mrs Debbie Nichols in the Text/Information Processing Center for the preparation of the manuscript. This study was supported in part by grants from the National Institutes of Health (AI 15231, AI 34577, AI 07047, and RR 585), and by grants from the Fisons Corporation, Rochester, NY, USA, and the Mayo Foundation.

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