

Ragweed-specific antibodies in bronchoalveolar lavage fluids and serum before and after segmental lung challenge: IgE and IgA associated with eosinophil degranulation

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Background: Migration of eosinophils and release of eosinophil degranulation products into bronchoalveolar lavage fluid is a consistent finding in studies of late responses to allergen challenge in the lung. However, the mechanism of eosinophil activation and release of eosinophil products in vivo is unclear.

Objective: We investigated the hypothesis that antigen-specific IgG, IgA, secretory IgA, or IgE is responsible for the eosinophil activation observed in the late-phase pulmonary reaction.

Methods: Ragweed-specific IgE, IgA, secretory IgA, and IgG were measured by monoclonal antibody-based immunoassays in bronchoalveolar lavage (BAL) fluid and in serum from 19 asthmatic subjects allergic to ragweed and six healthy nonallergic control subjects before and 20 hours after segmental lung challenge with ragweed extract. Eosinophil cationic protein (ECP) was also measured in BAL fluid as a marker of eosinophil activation.

Results: Most allergic asthmatic subjects had detectable levels of ragweed-specific IgE, IgA, and IgG in their serum and BAL fluid, whereas normal subjects had ragweed-specific IgA with no ragweed-specific IgE and little ragweed-specific IgG. IgA was the dominant ragweed-specific antibody isotype in BAL fluids. Ragweed-specific sIgA ($r_s = 0.52, p = 0.02$) and IgA ($r_s = 0.50, p = 0.03$) in BAL fluid after segmental lung challenge were significantly correlated with ECP. Ragweed-specific IgE and IgA in serum also correlated with ECP ($r_s = 0.74, p < 0.001$ and $r_s = 0.48, p = 0.04$, respectively).

Conclusions: The correlation of allergen-specific IgA and IgE antibody levels with ECP as a marker of eosinophil degranulation suggests an important role for IgE antibodies in allergic pulmonary inflammation and a potential role for antigen-specific IgA in eosinophil degranulation in the lung after antigen challenge. (*J Allergy Clin Immunol* 1998;101:265-73.)

Key words: Asthma, IgE, IgA, bronchoalveolar lavage, lung, eosinophil, ragweed

Abbreviations used

BAL:	Bronchoalveolar lavage
ECP:	Eosinophil cationic protein
PBS:	Phosphate-buffered saline
PNU:	Protein nitrogen units
sIgA:	Secretory IgA
SLC:	Segmental lung challenge

Allergic asthma is a disease characterized by airway inflammation during periods of allergen-induced bronchoconstriction.¹ An immediate phase of allergic inflammation occurs when allergen-specific IgE antibodies bound to the surface of mast cells and basophils are cross-linked by allergen.² Immunologic mechanisms involved in late-phase allergic responses that occur 8 to 24 hours after an acute reaction are, however, not well defined. One consistent finding in studies of late-phase responses in the lung is the migration of eosinophils and release of eosinophil degranulation products into the bronchoalveolar lavage (BAL) fluid.^{1,3,4} Eosinophil recruitment and degranulation have also been associated with increased bronchial vascular permeability after antigen challenge.⁴ Eosinophil degranulation products such as major basic protein are toxic to respiratory epithelial cells and can result in epithelial cell desquamation and tissue damage.^{5,6} In addition, eosinophil major basic protein also increases the reactivity of respiratory smooth muscle to cholinergic agonists.⁷

One potential mechanism for eosinophil degranulation is activation through the cell surface immunoglobulins. Receptors for IgA (Fc α R), IgE (Fc ϵ RI), and IgG (Fc γ RII) have been identified on the eosinophil surface.⁸⁻¹¹ Recent in vitro evidence suggests that eosinophils can be degranulated through their Fc α R and Fc γ RII.¹¹ Other in vitro work suggests that Fc α R are upregulated in atopic subjects.¹⁰ Additionally, the eosinophil expresses a receptor for a secretory component that suggests a role for secretory IgA (sIgA) antibody in the degranulation event.¹²

This study investigates the hypothesis that an antigen-specific immunoglobulin isotype is responsible for the eosinophil activation observed in the late-phase pulmonary reaction. We measured ragweed-specific IgA, sIgA, IgG, and IgE antibodies in BAL fluid and in serum

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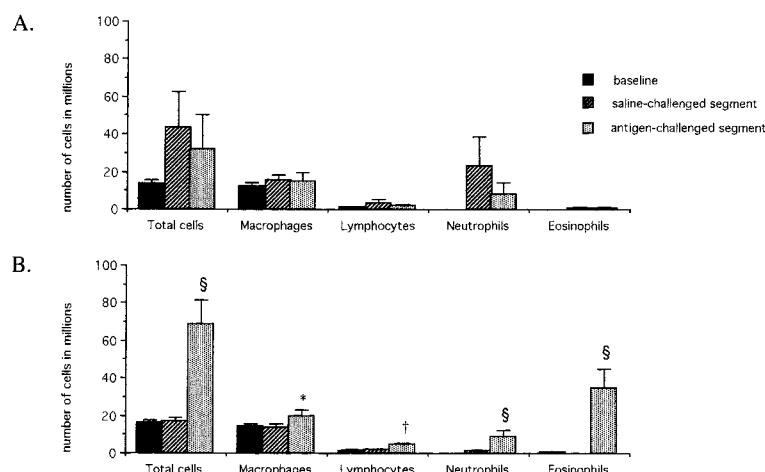


FIG. 1. Total number of cells in BAL fluid at baseline and after segmental saline and antigen challenges in normal control subjects (**A**, $n = 6$) and asthmatic subjects allergic to ragweed (**B**, $n = 19$). * $p < 0.05$, $^{\dagger}p < 0.01$, $^{\S}p < 0.001$, antigen-challenged segment greater than baseline or saline-challenged segments.

before and 20 hours after segmental lung challenge (SLC) with ragweed antigen. Strong correlations were observed that support the possibility that both ragweed-specific IgE and IgA antibodies are involved in the appearance of eosinophil degranulation products in the lungs of asthmatic subjects allergic to ragweed, and therefore may have a role in the mechanisms leading to pulmonary late-phase reactions.

METHODS

Subjects

The study subjects included men and women between the ages of 20 and 42 years with no smoking history. Allergic asthmatic subjects were characterized according to previously published criteria.¹ Allergic asthmatic subjects ($n = 19$) reported episodic wheezing and shortness of breath caused by seasonal or intermittent allergen exposure, exercise, and/or cold air exposure; had two or more positive skin test reactions to a battery of common inhaled antigens, including ragweed; and had a positive methacholine challenge test response.¹³ None of the subjects had ever received immunotherapy. Subjects had mild disease controlled with intermittent use of an inhaled β -agonist. No patients were taking inhaled corticosteroids, and inhaled β -agonist use was discontinued 1 day before the studies. Healthy control subjects ($n = 6$) had no rhinitic or asthmatic symptoms, negative skin test responses, and a negative methacholine challenge test response. All subjects were asymptomatic at the time of study, and FEV₁ was greater than 75% of predicted value.

SLC and BAL

Bronchoscopy was performed in accordance with guidelines recommended by a National Institutes of Health Workshop Committee.¹⁴ Subjects were premedicated with 0.6 mg of atropine and 0.1 mg of fentanyl administered intravenously. After inhalation of nebulized 4% lidocaine, an Olympus BF10 fiberoptic bronchoscope (Olympic Corp., Lake Success, N.Y.) was inserted into the lower airways. Local anesthesia was supplemented with 2% lidocaine. The tip of the bronchoscope was wedged into a subsegmental bronchus of the right upper

lobe, and BAL was performed to obtain baseline control measures. BAL was performed with five 20 ml aliquots of normal saline prewarmed to 37°C, with immediate aspiration of each aliquot. Next, a control sham challenge was performed by instilling 5 ml of normal saline into a subsegment of the lingula or right middle lobe. Ragweed antigen challenge was then performed by wedging a subsegment of the opposite lung (right middle lobe or lingula) followed by instillation of 5 ml of ragweed antigen at a concentration of 100 protein nitrogen units (PNU)/ml ($n = 10$) or 20 PNU/ml ($n = 9$). Control subjects were challenged with 5 ml of 1000 PNU/ml to assess nonspecific effects of ragweed antigen instillation. After 17 to 22 hours, a second bronchoscopy was performed with BAL at the site of the normal saline and antigen challenges. Fluids recovered from each site were pooled and processed separately for cell, mediator, and immunoglobulin measurements. Blood samples were collected at the time of the initial bronchoscopy for serum immunoglobulin measurements.

Differential cell counts

The volume of fluid recovered from each 100 ml lavage specimen was recorded, and the fluid was filtered through two layers of gauze. Before centrifugation, aliquots of fluid were removed for measurement of cell counts with a hemocytometer, cell viability was determined by trypan blue-dye exclusion, and differential cell counts were obtained by a Diff-Quik stain (American Scientific Products, McGaw Park, Ill.) of cytocentrifuge preparations (Cytospin; Shandon Southern Instruments, Inc., Sewickley, Pa.). A total of 500 cells were enumerated for each differential count during which macrophages, lymphocytes, neutrophils, eosinophils, and bronchial epithelial cells were enumerated.

Preparation of the BAL fluids for ragweed-specific antibody analyses

The BAL fluids (4 to 6 ml) were concentrated to 600 ml with a Centricon-10 filtration device (W. R. Grace & Co., Beverly, Mass.) before analysis for ragweed-specific IgA, IgG, and IgE antibody. After assay of concentrated BAL, results were expressed as nanograms per milliliter of unconcentrated BAL fluid. To evaluate potential losses of ragweed-specific IgA as a

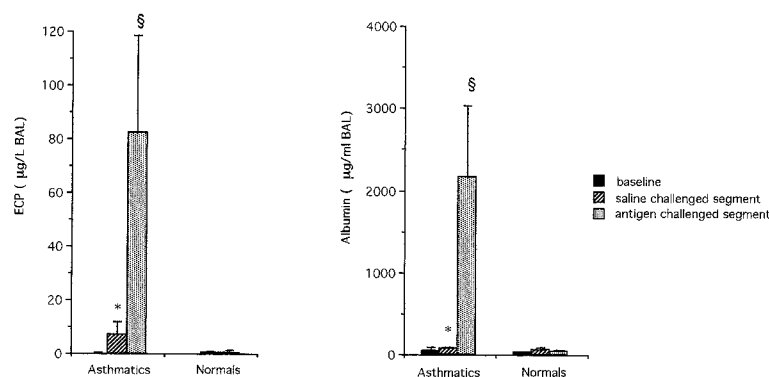


FIG. 2. ECP and albumin measurements in BAL fluid at baseline and after segmental saline and antigen challenge in normal control subjects ($n = 6$) and asthmatic subjects allergic to ragweed ($n = 19$). * $p < 0.01$, saline-challenged segment greater than baseline; § $p < 0.001$, antigen-challenged segment greater than baseline or saline-challenged segment.

result of the concentration procedure, one BAL fluid from this study with 717 ng/ml of ragweed-specific IgA was used undiluted and after redilution of a 10-fold concentrated sample. There was approximately a 20% loss in IgA anti-ragweed antibody as a result of the Centricon-based concentration process.

Reference standard for ragweed-specific IgA and sIgA

Standardization of the ragweed-specific IgA antibody assays was accomplished by using the BAL fluid with the highest observed antibody level as a reference that was calibrated by depletion analysis. In this assay, two 1 ml aliquots of the reference BAL fluid were separately incubated in duplicate with prewashed ragweed-conjugated Sepharose or unconjugated Sepharose (sham control) (0.5 ml of 50% vol/vol, rotated overnight at 4° in Eppendorf microtubes). After rotation, the two microtubes were centrifuged and the supernatants were removed and stored separately. Immunoassays were then performed to accurately determine the quantity of total IgA and total sIgA in the sham and ragweed Sepharose-absorbed reference preparations. Analysis on ragweed-coated microtiter plates showed that 97.6% of the ragweed-specific IgA and 99.3% of the ragweed-specific sIgA had been removed by rotation with ragweed Sepharose. Differences in the total IgA and total sIgA concentrations between the sham and ragweed Sepharose-absorbed BAL fluid generated an IgA anti-ragweed and sIgA anti-ragweed estimated at 1.26 µg/ml and 1.06 µg/ml, respectively.

Assays for ragweed-specific and total IgA and sIgA

Ragweed (same lot as used for depletion analysis) was coated onto microtiter plates (Immulon IV Removawells; Dynatech, McLean, Va.) at a final concentration of 50 µg/ml in phosphate-buffered saline (PBS; 0.1 ml/well for 16 hours at 4° C). Each plate was blocked with PBS-1% bovine serum albumin (30 minutes) and washed with PBS-0.05% Tween four times. All wells were then prefilled with 50 µl of PBS-1% bovine serum albumin assay buffer to prevent dryness during the pipetting of test specimens, and BAL fluid specimens were pipetted into their respective wells (50 µl/well). All plates were incubated overnight at 23° C. Plates were rewashed and 0.1 ml of detection antibody (e.g., biotin-labeled monoclonal anti-human

IgA-Fc [clone HP6123] or biotin-labeled monoclonal anti-human secretory component [clones HP6130/HP6141]) at 1 µg/ml was pipetted into their respective wells in the plate and incubated for 1 hr at 23° C.¹⁵ The specificity of these anti-IgA and anti-secretory component monoclonal antibodies have been documented elsewhere in an International Union of Immunological Societies-World Health Organization collaborative study.¹⁶ After a buffer wash, streptavidin-horseradish peroxidase (0.1 ml/well, 1 µg/ml) was pipetted into all wells in the plate and incubated for 1 hr at 23° C. After the final buffer wash, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-H₂O₂ substrate was added and the color reaction developed until the absorbance of the highest standard at 405 nm reached 1.5 to 2.0. The enzymatic reaction was stopped with 2 mmol/L sodium azide, and optical density readings were obtained with a microtiter plate reader (Dynatech MR4000, McLean, Va.). To ensure specificity of the antibody measurements for ragweed, an inhibition assay was performed in the same plate by preincubating 50 µl of antibody-containing test fluid with 50 µl of 50 mg/ml ragweed instead of buffer. The concentration of the antibody measured in the inhibition assay was subtracted from the total antibody level to compute the concentration of the ragweed-specific IgA in that particular fluid. The lower limit of detection of the ragweed-specific IgA and sIgA assays in this study were 4.3 ng/ml and 8.3 ng/ml, respectively. Results are expressed in nanograms per milliliter of unconcentrated BAL fluid.

Total IgA and sIgA levels in BAL fluid were measured by monoclonal antibody-based immunoassays as previously reported.¹⁵ For the total IgA immunoassay, murine monoclonal antibody 6111P was used as the capture antibody, and both murine monoclonal antibodies 6141P and 6130P were used in combination for capture antibodies to measure sIgA. In both the total IgA and sIgA, the detection antibody was murine monoclonal antibody 6123B.

Assays for ragweed-specific IgE in serum and BAL fluid

The assay used for ragweed-specific IgE measurement is a modification of a previously reported method.¹⁷ Briefly, 0.1 ml of serum or concentrated BAL fluid was incubated in 12 × 75 mm test tubes with 0.5 ml of Sepharose 4B-CL beads (3% vol/vol) that had been previously coupled with the same ragweed allergen used in skin testing. A buffer control and eight dilutions of an IgE anti-ragweed-containing reference serum

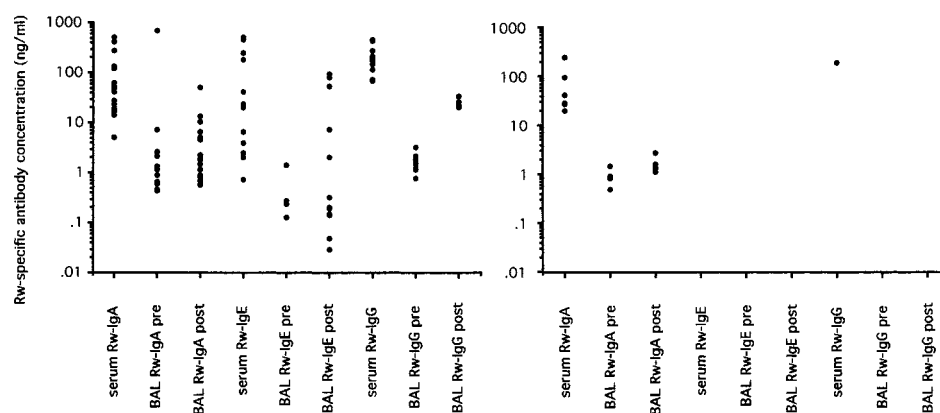


FIG. 3. Concentration of ragweed-specific IgE, IgA, sIgA, and IgG antibodies in serum and BAL fluid from both asthmatic subjects allergic to ragweed (*left panel*, $n = 19$) and normal control subjects (*right panel*, $n = 6$). *Rw*, Ragweed.

were analyzed in triplicate. The standards and sample tubes were rotated at 23°C overnight, washed three times with 3.0 ml of buffer, and labeled with 0.5 ml of ^{125} I-labeled polyclonal anti-human IgE. After a final orbital rotation and buffer wash, bound radioactivity was counted to statistical precision in a γ -scintillation counter. IgE anti-ragweed results were interpolated from the calibrated reference serum curve; the lower limit of detection of the assay was 0.68 ng/ml. Results are expressed in nanograms per milliliter of unconcentrated BAL fluid.

Assays for ragweed-specific IgG in serum and BAL fluid

The assay for IgG anti-ragweed is similar to the IgE anti-ragweed assay with the following exceptions. All sera and BAL fluids were analyzed at a 1:40 and 1:6 dilution, respectively, after preabsorption with uncoupled agarose to remove naturally occurring carbohydrate-specific IgG antibody.¹⁷ Second, ^{125} I-Staphylococcal Protein A was used to detect bound IgG antibody. Finally, response results were interpolated from an IgG anti-ragweed standard curve that was independently calibrated by depletion analysis.¹⁸ The minimal detectable IgG anti-ragweed concentration in the assay was 50 ng/ml after correction for dilution. Results are expressed in nanograms per milliliter of unconcentrated BAL fluid.

Measurement of eosinophil cationic protein

Eosinophil cationic protein (ECP) was measured in unconcentrated BAL fluids by using the Pharmacia CAP System (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) according to instructions in the package insert. The lower limit of detection for that assay was 0.54 ng/ml.

Statistical analyses

The Wilcoxon matched-pairs test was used to compare BAL results obtained with saline and antigen challenges within the normal and asthmatic groups. The Mann-Whitney U test was used to compare unpaired results between groups. Correlations were determined by Spearman rank analysis. In correlating mean antibody levels for each subject group, values below the limit of detection were assigned a value that was half the lower limit of detection for that assay. A repeated measure analysis of variance was used to assess the significance of changes in total cell numbers collected in the subjects' BAL fluid at the baseline, normal saline challenge, and antigen challenge sites. Probability

(p) values less than 0.05 were considered significant. All computations were performed using SPSS for Windows, Base System, Release 5.0.1 (SPSS, Chicago, Ill.).

RESULTS

Subject characteristics

Six healthy control subjects and 19 asthmatic subjects allergic to ragweed underwent the study protocol. The ages of the control and asthmatic groups were comparable (29.7 ± 1.4 and 31.3 ± 2.4 years [mean $\pm$ SEM], respectively). The male to female ratio was 2:4 in the control group and 10:9 in the asthmatic group. All asthmatic subjects responded to methacholine at a concentration less than 5 mg/ml. Normal subjects had no response at the highest concentration of 25 mg/ml.

Inflammatory cell responses in BAL fluid

Enumeration of BAL cells in normal and asthmatic subjects before and after SLC are shown in Fig. 1. Fluid recovery at each site averaged $56.3\% \pm 4.0\%$ in control subjects and $44.8\% \pm 2.6\%$ in asthmatic subjects. Fluid recoveries were not significantly affected in the antigen-challenged sites. A neutrophil response was seen occasionally in normal subjects, but was not specific to antigen as the response was also seen at sites of saline challenge. In allergic asthmatic subjects, there was about a fourfold increase in the total number of cells recovered by BAL at sites of antigen challenge compared with baseline and saline-challenged segments. Macrophages ($p < 0.05$), lymphocytes ($p < 0.01$), neutrophils ($p < 0.001$), and eosinophils ($p < 0.001$) increased in the ragweed-challenged segment compared with both the baseline BAL and saline-challenged sites. The most striking change was in the number of eosinophils in the antigen-challenged segment, which increased 174-fold in comparison with the saline-challenged site. Additionally, there were significantly more eosinophils in the baseline BAL of the asthmatic subjects compared with the control subjects ($p < 0.001$).

TABLE I. Concentrations of ragweed antibodies measured in serum and BAL fluids

	Asthmatic subjects		Normal subjects	
	No. of patients with detectable levels	Mean $\pm$ SEM (ng/ml)	No. of patients with detectable levels	Mean $\pm$ SEM (ng/ml)
Ragweed-specific IgE				
Serum	14/18	91.8 $\pm$ 36.1	0/6	
Baseline BAL	6/19	<0.5	0/6	
Post-SLC BAL	11/19	12.7 $\pm$ 6.8	0/6	
Ragweed-specific IgA				
Serum	18/18	103.9 $\pm$ 33.2	6/6	73 $\pm$ 35
Baseline BAL	13/19	36.8 $\pm$ 35.8	4/6	1 $\pm$ 0.2
Post-SLC BAL	19/19	5.6 $\pm$ 2.7	6/6	2 $\pm$ 0.2
Ragweed-specific sIgA				
Post-SLC BAL	10/19	3.2 $\pm$ 1.3	5/6	2 $\pm$ 1
Ragweed-specific IgG				
Serum	10/18	127.9 $\pm$ 34.5	1/6	52 $\pm$ 27
Baseline BAL	9/19	1.1 $\pm$ 0.2	0/6	
Post-SLC BAL	5/19	3.75	0/6	

Individual values below the limit of detection were assigned a value that was half the lower limit of detection for that assay in calculations performed to determine mean and SEM.

ECP and albumin in BAL fluid

Fig. 2 shows the marked increases in ECP and albumin in BAL fluid after segmental antigen challenge in the asthmatic subjects. ECP levels in baseline BAL fluids were almost universally undetectable. There were significant increases in the BAL fluid concentrations of ECP and albumin in the antigen-challenged segment ($p < 0.001$) compared with both the baseline and the saline controls. ECP and albumin were strongly correlated at sites of antigen challenge ($r_s = 0.72$, $p < 0.001$). There was also a small but statistically significant increase in the concentrations of ECP ($p < 0.01$) and albumin ($p < 0.001$) in the BAL fluid from the saline-challenged segment compared with baseline in the asthmatic subjects, indicating some inflammatory reaction at a site not specifically challenged with antigen. No response was observed in normal subjects. These results show the specificity of the antigen-induced inflammation in subjects who are appropriately sensitized.

Ragweed-specific antibody levels in serum and BAL fluid

Ragweed-specific IgE, IgA, sIgA, and IgG antibody concentrations were quantified in both the serum and the BAL fluid from both the asthmatic subjects allergic to ragweed and the normal control subjects. Data are presented in Fig. 3. Not all subjects had detectable ragweed-specific antibody levels, and the number of subjects with detectable antibody levels are presented in Table I. The means of the ragweed-specific antibody levels are also presented in Table I. Subjects who had undetectable antibody levels were assigned an antibody concentration that was half that of the lower limit of detection for that particular assay in calculating the mean values shown in Table I. The lower limits of detection are given in the Methods section.

In serum, both asthmatic and control subjects had similar levels of ragweed-specific IgA, whereas ragweed-specific IgG was less frequent in control than asthmatic subjects. As expected, on the basis of skin test reactivity, ragweed-specific IgE was only detected in the allergic asthmatic group.

In baseline BAL before allergen challenge, asthmatic subjects had significantly higher ragweed-specific IgE and IgG levels than normal control subjects. Ragweed-specific IgA was similar in both groups. After SLC, significant increases in ragweed-specific IgE, IgG, and IgA were observed at sites of allergen challenge in the asthmatic subjects. Although the mean level of ragweed-specific IgA at baseline was greater than that after SLC, this reflects the contribution of two outlying values at baseline. When compared by nonparametric statistics, ragweed-specific IgA concentrations after SLC demonstrated significant increases compared with baseline measurements. No significant changes in ragweed-specific immunoglobulins in BAL fluid were observed in normal subjects after SLC.

To assess possible relationships between serum and BAL antibody levels, correlations were sought before and after SLC within the allergic asthmatic group (Table II). There were significant correlations between the serum levels of ragweed-specific IgE and the BAL levels of ragweed-specific IgE both before and after SLC. These same significant correlations were also true for serum and BAL levels of ragweed-specific IgA, but not for ragweed-specific IgG.

The ratio of ragweed-specific IgA to total IgA in baseline BAL fluid and in serum was determined in both the asthmatic and control subjects. Ragweed-specific IgA composed 0.01% of total IgA in both BAL fluid and serum of both asthmatic and normal subjects. Despite similar ratios of specific and total IgA levels, the ratio of ragweed-specific IgA to total IgA in BAL fluid at

TABLE II. Spearman rank correlations between ragweed-specific immunoglobulin levels in serum and BAL fluids in asthmatic subjects allergic to ragweed

	Serum ragweed antibody levels		
	IgE	IgA	IgG
BAL ragweed antibody levels			
Baseline	$r_s = 0.54$ $p = 0.02$	$r_s = 0.65$ $p = 0.004$	$r_s = 0.28$ $p = 0.25$
Post-SLC	$r_s = 0.56$ $p = 0.02$	$r_s = 0.84$ $p < 0.001$	$r_s = 0.41$ $p = 0.09$

N = 19.

baseline did not correlate with the ratio of ragweed-specific IgA to total IgA in serum in either the asthmatic ($r_s = 0.14$; $p = 0.3$) or normal ($r_s = 0.63$; $p = 0.1$) subjects. This suggests that the amount of ragweed-specific IgA in BAL fluid is not solely a result of plasma exudation but may be influenced by local production.

When total IgA and total sIgA levels in BAL fluids of allergic asthmatic subjects were compared before and after SLC, total IgA increased from 15.1 ± 2 to 128.2 ± 57 $\mu\text{g/ml}$ BAL (mean $\pm$ SEM, $n = 19$, $p < 0.001$), whereas sIgA increased from 12.2 ± 2.7 to 18.7 ± 3.6 $\mu\text{g/ml}$ BAL ($p < 0.03$). These changes provide further evidence of plasma influx contributing to IgA in BAL fluid after SLC, and the larger increase in total IgA compared with the increase in sIgA likely reflects the composition of IgA in plasma in the unconjugated form.

ECP in BAL fluid

Analyses were also performed to determine if correlations existed between ECP found in BAL fluid after SLC and antibody levels (Table III). The strongest correlation with ECP measurements in BAL fluid after ragweed SLC was ragweed-specific IgE in serum ($r_s = 0.74$, $p < 0.001$). Ragweed-specific IgE in BAL fluid after SLC was also strongly correlated ($r_s = 0.64$, $p = 0.003$) with ECP. These correlations between ECP and ragweed-specific IgE were stronger than the correlations between ECP and the net influx of eosinophils into the BAL fluid as a result of SLC ($r_s = 0.57$, $p = 0.01$) and the total number of eosinophils found in the BAL fluid after SLC ($r_s = 0.62$, $p = 0.005$). Ragweed-specific sIgA ($r_s = 0.52$, $p = 0.02$) and IgA ($r_s = 0.50$, $p = 0.03$) measured in BAL fluid after SLC also significantly correlated with ECP in the same fluids. Serum ragweed-specific IgA correlated ($r_s = 0.48$, $p = 0.04$) with BAL fluid ECP levels after SLC, but there were no significant correlations between either serum or BAL ragweed-specific IgG and ECP. Analyses were also performed to determine if correlations existed between total eosinophils found in BAL fluid after SLC and antibody levels (Table IV). Serum ragweed-specific IgE correlated with eosinophil influx. However, the correlation observed between ragweed-specific IgA in both serum and BAL fluid after

SLC with ECP was not observed when these immunoglobulin levels were correlated with total eosinophil numbers. This finding suggests a role for IgA in eosinophil activation.

DISCUSSION

Recent studies have implicated IgA as a contributor to allergic inflammation.^{10, 11} However, the in vivo relevance of these in vitro findings has not been confirmed. In this report we developed new methods by using highly specific monoclonal antibodies to quantify antigen-specific sIgA in BAL fluid and antigen-specific IgA and in both BAL fluid and serum.¹⁵ Our techniques used ragweed antigen as a capture protein, therefore ragweed-specific immunoglobulins that formed immune complexes with ragweed allergen used for challenge are probably not detected in our assay. We also used previously described methods to measure ragweed-specific IgE and IgG in the same fluids. The reason for performing these measurements was to address the question of how the eosinophil may be stimulated to degranulate in vivo during an allergic reaction. As a marker of eosinophil degranulation, we quantified ECP and hypothesized that if activation occurred through an immunoglobulin-mediated mechanism, there might be a correlation between antigen-specific antibody levels and evidence of eosinophil degranulation.

In this study we found that ragweed-specific IgE most strongly correlated with ECP release. Both ragweed-specific IgE in serum and in the post-SLC BAL fluid strongly correlated with the appearance of ECP in BAL fluid after SLC. Although IgE has long been known to be a critical participant in the allergic inflammatory response, the role of antigen-specific IgE in eosinophilic inflammation has not been fully elucidated. In the immediate-phase reaction, IgE activates mast cells through antigen-specific mechanisms. These cells may produce granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5, all of which have important eosinophil regulatory functions.¹⁹⁻²³ These cytokines are involved in eosinophil recruitment, survival, and priming. The role of mast cell involvement in eosinophil recruitment has been shown in a recent animal study, which revealed that allergen challenge in sensitized, mast cell-deficient mice resulted in significantly fewer eosinophils in the lung tissue and BAL fluid than in normal congenic litter mates.²⁴ Although it has been reported that IgE receptors are present on eosinophils,^{8, 9} IgE has not been shown to cause eosinophil degranulation in vitro,²⁵ and it may be more likely that IgE is involved in eosinophil recruitment and priming than in direct activation of eosinophils. A recent clinical study by Fahy et al.²⁶ confirmed the importance of IgE in asthmatic late-phase, physiologic responses in which the eosinophil may play an important contributory role. That study showed that treatment of allergic asthmatic subjects with a humanized murine monoclonal antibody directed against IgE significantly reduced the mean maximal fall in FEV₁ during the late response. BAL was

TABLE III. Spearman rank correlations between ragweed-specific immunoglobulins and ECP levels in BAL fluid after SLC in asthmatic subjects

	Ragweed-specific IgE	Ragweed-specific IgA	Ragweed-specific sIgA	Ragweed-specific IgG
Serum	$r_s = 0.74$ $p < 0.001$	$r_s = 0.48$ $p = 0.04$	ND	$r_s = -0.05$ $p = 0.84$
Baseline BAL	$r_s = 0.31$ $p = 0.01$	$r_s = 0.39$ $p = 0.1$	ND	$r_s = -0.043$ $p = 0.06$
Post-SLC BAL	$r_s = 0.64$ $p = 0.003$	$r_s = 0.50$ $p = 0.03$	$r_s = 0.052$ $p = 0.02$	$r_s = 0.4$ $p = 0.09$

ND, Not done.

TABLE IV. Spearman rank correlations between ragweed-specific immunoglobulins and total eosinophils in BAL after SLC in asthmatic subjects allergic to ragweed

	Ragweed-specific IgE	Ragweed-specific IgA	Ragweed-specific IgG
Serum	$r_s = 0.63$ $p < 0.005$	$r_s = 0.24$ $p = 0.34$	$r_s = -0.21$ $p = 0.41$
Baseline BAL	$r_s = -0.09$ $p = 0.74$	$r_s = 0.02$ $p = 0.93$	$r_s = -0.46$ $p = 0.05$
Post-SLC BAL	$r_s = 0.57$ $p = 0.01$	$r_s = 0.31$ $p = 0.20$	$r_s = 0.39$ $p = 0.10$

N = 19.

not done in these patients so the effect of the anti-IgE antibody on the appearance of eosinophil products in the late phase is unknown, but one would predict that eosinophilic inflammation would be decreased by this treatment.

BAL fluids from asthmatic subjects allergic to ragweed can lead to degranulation of eosinophils in vitro, and the degree of degranulation correlates with the levels of antigen-specific IgA, IgG, and IgG₁ found in those fluids.¹¹ These results suggest that these immunoglobulin isotypes may mediate eosinophil degranulation in an antigen-specific fashion. In our study neither the concentrations of ragweed-specific IgG in serum or BAL fluid from either before or after SLC correlated with ECP release. In fact, there were weak negative correlations between these IgG levels and ECP. However, we did find significant correlations between the levels of ECP in BAL fluid after SLC and the levels of ragweed-specific IgA both in serum and in the BAL fluid after SLC. There was also a significant correlation between ragweed-specific sIgA present in BAL fluid after SLC and the levels of ECP from the same fluid. There was no correlation between the concentrations of ragweed-specific IgA in the pre-SLC BAL fluids and ECP. It is unlikely that an immediate increase in the local production of ragweed-specific IgA occurred within the 20-hour time frame after SLC, and plasma leakage of ragweed-specific IgA into the BAL fluid after the initial inflammatory response is likely to account for the change in the concentration of ragweed-specific IgA in the BAL fluid after SLC. This explanation is supported by the increases in ragweed-specific IgE and IgG, as well as albumin and total IgA observed after SLC, consistent with significant

plasma exudation occurring during the inflammatory response.

The significant correlation between the increase in an eosinophil degranulation product in BAL fluid after SLC and the increase in albumin concentration in the same fluids has been previously reported^{4,27} and suggests that eosinophil granule products play a role in the altered airway permeability observed with SLC. It is likely that eosinophil activation occurs most intensely at the sites of eosinophil accumulation in the lung, and our results suggest that allergen binding by specific immunoglobulins on cells recruited to the airway is a possible mechanism by which eosinophil degranulation may occur. IgA, particularly sIgA, has been shown to mediate eosinophil degranulation in vitro,²⁸ and increased expression of IgA receptors on eosinophils of allergic individuals has been reported.¹⁰ These observations support a role for IgA-mediated mechanisms for eosinophil activation in allergic airway inflammation. There was not a correlation between ragweed-specific IgA levels in either the serum or in the BAL fluid in the antigen-challenged segment and total eosinophil numbers, suggesting that antigen-specific IgA is not involved in eosinophil recruitment.

ECP measurements in serum were not performed in this study, and previous work has shown that ECP levels in serum correlated with those found in BAL fluid after antigen challenge.²⁹ Venge³⁰ has hypothesized that activation of eosinophils may occur in both the blood and the lung, suggesting the possibility that circulating ECP may contribute to increases in tissue ECP at inflammatory sites. In one study serum levels of ECP in mild-to-moderate asthmatic subjects not taking antiinflamma-

tory medications was 49.6 ± 53.5 $\mu\text{g/L}$, whereas BAL fluid concentrations in the same subjects were 15.6 ± 9.0 $\mu\text{g/L}$.²⁹ The similarity between the serum and BAL fluid ECP concentrations and the large dilutional factors inherent in BAL fluid support the idea of local release of ECP into the airways. The present data, which show a mean ECP concentration of about 80 $\mu\text{g/L}$ in the SLC model, also support this possibility.

The observed correlations of ECP with ragweed-specific IgA and sIgA in BAL fluids support the concept that ragweed-specific IgA may be involved in eosinophil degranulation. One possible explanation for these findings would be that ragweed-specific IgE mechanisms are responsible for eosinophil migration into the allergen-challenged tissues, whereupon ragweed-specific IgA may initiate the actual degranulation process. It is necessary to acknowledge the limitations of such correlation analyses and to realize that they do not demonstrate a definitive cause and effect relationship. It is difficult to know whether the increase in immunoglobulins in BAL fluid is the cause or the result of eosinophilic inflammation. Furthermore, the immunoglobulin levels in BAL fluid may be affected by both serum levels of immunoglobulins and degree of airway inflammation. We are presently pursuing other studies to dissect more definitively the role of antigen-specific IgA in allergen-induced inflammation in general, and in the late-phase allergic reaction in particular.

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