

Antigen-specific IgE and IgA antibodies in bronchoalveolar lavage fluid are associated with stronger antigen-induced late phase reactions

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Summary

Background The mechanism(s) leading to the development of late phase allergic reactions is (are) unknown. Previous studies have indicated that a relationship between serum IgE and the late phase exists.

Objective To explore the relationships between allergen-specific immunoglobulins in bronchoalveolar lavage (BAL) fluids and the magnitude of airflow limitation during the late phase response to inhaled allergen.

Methods Ragweed-specific IgE, IgA, secretory IgA (sIgA) and IgG were measured in BAL fluid and in the serum 1–5 weeks before whole lung antigen challenge with ragweed extract, in 16 ragweed allergic asthmatics. In addition, BAL and serum eosinophil cationic protein (ECP) and BAL fibrinogen levels were determined and BAL cells counted and differentiated. The latter procedures were repeated in a second BAL performed 24 h after the end of the ragweed challenge. After the challenge, lung function was monitored hourly for 8 h, to record the magnitude of airflow limitation.

Results Ragweed-specific immunoglobulins were detected in 25% to 37.5% of BAL samples. Compared to the subjects with undetectable BAL fluid ragweed-specific IgE levels at baseline, those with detectable antibodies had stronger late phase reactions as determined by the nadir of FEV₁ between hours 4 and 8 after the ragweed inhalation challenge ($P = 0.0007$). Allergen-induced changes in BAL ECP and fibrinogen levels were also higher in those subjects with detectable ragweed-specific IgE in baseline fluids ($P = 0.03$ and $P = 0.005$, respectively). Significant relationships between BAL antigen-specific IgA, serum ragweed-specific IgE and IgA and the late phase reaction were also found.

Conclusion The results of this study point towards the possibility that allergen-specific IgE and IgA may be independently involved in the pathogenesis of the late phase reaction. This notion merits further exploration.

Keywords: IgE, IgA, sIgA, asthma, allergen challenge, bronchoprovocation, ragweed, fibrinogen, ECP, eosinophils

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Introduction

In examining the mechanisms of the late phase allergic reaction, we have developed an interest in the potential role of IgE, IgA, sIgA and even IgG in mediating the activation of various cells that participate in allergic inflammation. In

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addition to mast cells and basophils, receptors for IgE have been identified on eosinophils [1–3], which appear to play a central role in allergic inflammation, and on monocytes/macrophages [4,5]. The eosinophil also carries receptors for IgA (Fc α R) and IgG (Fc γ RII) [6,7], which can mediate cell degranulation. Finally, an eosinophil receptor for the secretory component has been detected; this may be involved in preferential degranulation mediated by sIgA [8].

To achieve our goal, we have developed methodologies to quantify allergen-specific immunoglobulin levels in bronchoalveolar lavage (BAL) fluids [9]. Using the experimental model of segmental allergen challenge in patients with asthma, we recently detected a positive correlation between the baseline levels of ragweed-specific IgE and IgA in BAL fluids and the ragweed-induced change in ECP, a marker of eosinophil activation, 18 h after the challenge [10]. Given the strong relationship between eosinophil accumulation/activation and the physiological manifestations of the late phase reaction [11,12], we designed the current protocol with the hypothesis that baseline BAL fluid ragweed-specific IgE, and possibly IgA or sIgA, relate to the late reduction in lung function that is generally observed in 30–50% of asthmatics undergoing a conventional 'whole lung' allergen inhalation challenge.

In both our and other investigators' hands, using standard methodology, whole lung antigen inhalation produces consistent intrasubject pulmonary late phase reactions [13,14]. In conjunction with the physiological manifestations of the late phase, we chose to collect information on some cellular and humoral aspects of the late allergic response, namely BAL leucocyte counts, and the levels of ECP and fibrinogen. Fibrinogen is a large intravascular protein, the appearance of which in BAL indicates plasma extravasation in a more specific and possibly more sensitive manner compared to albumin [15]. To examine whether any detectable relationships can be specifically attributable to the BAL immunoglobulins, we also quantified their serum counterparts.

Methods

Subjects

We recruited 16 allergic asthmatic subjects with no smoking history from the pool of volunteers at the Johns Hopkins Asthma and Allergy Center Experimental Pulmonary and Nasal Physiology Laboratory. The average age was 28.6 (range: 20–41). Seven subjects were female. All subjects reported episodic wheezing and shortness of breath to seasonal or intermittent allergen exposure, exercise, and/or cold air exposure and had a positive

(> 20% reduction in FEV₁) methacholine challenge to a concentration less than or equal to 8 mg/mL. None were on inhaled corticosteroids and inhaled beta-agonist usage was discontinued 1 day before the studies. All subjects were asymptomatic at the time of the study. Atopic status was determined by skin prick testing with a battery of nine common inhalant allergens. All subjects had a positive skin test to ragweed. Most subjects were also positive to various other aeroallergens.

Study protocols

The study protocols were approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center and all subjects gave written consent. On visit 1, between 10 a.m. and 12 p.m., subjects underwent bronchoscopy with lavage of a right upper lobe subsegment. One to 5 weeks later, the subjects returned to the laboratory for visit 2 of the study and underwent a whole lung antigen challenge with ragweed extract (Greer Laboratories Inc., Lenoir, NC). On this visit, each subject came to the laboratory at 8 a.m. and pulmonary function testing was performed to ensure that FEV₁ was greater than 60% of predicted. The subject then underwent antigen challenge by inhalation which involved first a diluent provocation (phosphate buffered saline) followed by half-log increasing concentrations of ragweed extract. The allergen provocation was terminated when at least a 20% reduction in FEV₁ from the values obtained after the diluent had occurred. From the interpolation of the FEV₁-based dose–response curve to the allergen challenge, the cumulative dose of allergen in breath units (1 breath unit = 1 breath of a 1 PNU/mL allergen solution) causing a 20% reduction in FEV₁, logarithmically transformed for analysis, yielded the log antigen PD₂₀. After the end of the challenge, subjects were monitored with hourly spirometry for 8 h to record the magnitude of the late phase reaction.

On visit 3, 24–26 hours after the end of the whole lung antigen challenge, a second bronchoscopy was performed with bronchoalveolar lavage (BAL) at the previous right upper lobe bronchoscopy site. To avoid confounding effects by possible diurnal variations, the bronchoscopy performed on visit 3 took place at the same time of the day that the visit 1 bronchoscopy had been performed. BAL fluids and blood samples were collected at the time of each bronchoscopy for cell counts and for measurement of immunoglobulins and other biochemical markers.

Bronchoscopy was performed in accordance with guidelines recommended by a National Institutes of Health Workshop Committee [16]. Subjects were pre-medicated intravenously with atropine 0.6 mg and fentanyl 0.1 mg. Following inhalation of nebulized 4% lidocaine, an Olympus BF10 fiberoptic bronchoscope (Olympus Corp.,

Lake Success, NY) was inserted into the lower airways. Local anaesthesia was supplemented with 2% lidocaine. The tip of the bronchoscope was wedged into a subsegmental bronchus of the right upper lobe and bronchoalveolar lavage was performed with five 20 mL aliquots of normal saline, pre-warmed to 37 °C, with immediate aspiration of each aliquot. The total fluid recovered was pooled, centrifuged, and processed separately for cellular and biochemical analysis.

Cell counts and differentials

The volume of fluid recovered from each 100-mL lavage was recorded, and the fluid was filtered through two layers of gauze. Before centrifugation, aliquots of fluid were removed for cell counts by haemocytometer, cell viability by trypan blue dye exclusion, and differential cell counts by Diff-Quick® staining (American Scientific Products, McGaw Park, IL) of cytocentrifuge preparations (Cytospin®, Shandon Southern Instruments, Inc., Sewickley, PA). A total of 500 cells were enumerated for differential counts, which identified macrophages, lymphocytes, neutrophils, eosinophils and bronchial cells.

Preparation of the BAL fluids for ragweed-specific antibody analyses

Ten ml of the recovered BAL fluid from each subject was concentrated to 1 mL using Centricon-10 (W.R. Grace & Co., Beverly, MA) filters prior to analysis for ragweed-specific IgE, IgA, sIgA and IgG antibody.

Assays for ragweed-specific IgA (in serum and BAL) and sIgA (in BAL)

Lyophilized ragweed was diluted into sterile water to a concentration of 50 µg/mL and this solution was coated onto microtiter plates (0.1 mL/well, 16 h at 4 °C) (Immulon IV Removawells, Dynatech, McLean, VA). Each plate was blocked and washed with PBS-0.05% Tween. All wells were pre-filled with 50 µL of PBS-1% BSA 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 re-washed and 0.1 mL of detection antibody (biotin-labelled antihuman IgA-Fc (HP6123) or biotin labelled antihuman secretory component (HP6130/HP6141)), at 1 µg/mL, was pipetted into the respective wells and incubated 1 h at 23 °C [9]. After a buffer wash, streptavidin-horseradish peroxidase (0.1 mL/well, 1 µg/mL) was pipetted into all wells and incubated for 1 h at 23 °C.

Following the final buffer wash, the ABTS substrate was added and developed until the absorbance of the highest standard at 405 nm reached 1.5–2.0. The enzymatic reaction was stopped with 2 mM sodium azide and absorbance readings were obtained in a microtiter plate reader (Dynatech MR4000, McLean, VA). To ensure specificity of the measurements, an inhibition assay was performed in the same plate by pre-incubating 50 µL of antibody containing test fluid with 50 µL of 50 mg/mL of ragweed solution (dissolved in sterile water) instead of buffer. The concentration of the antibody measured in the inhibition assay was subtracted from the total antibody level to give the concentration of ragweed-specific IgA in that particular fluid. The lower limit of detection for ragweed-specific IgA in this assay and in this study was 4.3 ng/mL; for ragweed-specific sIgA, the lower limit of detection was 8.3 ng/mL. The calibration of the reference standard ragweed-specific IgA and sIgA antibodies has already been described [10].

Assays for ragweed-specific IgE in serum and BAL fluid

The assay employed for ragweed-specific IgE measurement has been previously described [17]. Briefly, 0.1 mL of the serum or concentrated BAL fluid was incubated in 12- by 75-mm test tubes with 0.5 mL of Sepharose 4B-CL beads that had been previously coupled with ragweed allergen. A buffer control and an eight dilution reference curve in triplicate were constructed using a calibrated antiragweed IgE containing serum. The tubes were rotated at 23° overnight, washed three times with 3.0 mL of buffer, and labelled with 0.5 mL of I¹²⁵-labelled antihuman IgE. Following a final orbital rotation and buffer wash, bound radioactivity was counted to statistical precision in a gamma scintillation counter. Antiragweed IgE results were interpolated from the reference serum which displayed a lower limit of detection of 0.68 ng/mL.

Assays for ragweed-specific IgG in serum and BAL fluid

The assay for antiragweed IgG is similar to that for antiragweed IgE with the following exceptions. First, all sera and BAL fluids were analysed at a 1 : 40 and 1 : 6 dilution, respectively, following pre-absorption with uncoupled agarose to remove naturally occurring carbohydrate-specific IgG antibody [17]. Second, I¹²⁵ Staphylococcal Protein A was used to detect bound IgG antibody. Finally, response results were interpolated from an antiragweed IgG standard curve that was constructed with serum calibrated by depletion analysis as discussed for the antiragweed IgE reference serum. The minimal detectable antiragweed IgG concentration in the study was 50 ng/mL.

Measurement of ECP

The Pharmacia ECP assay kit for the CAP System was kindly provided by the manufacturer (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) and performed according to instructions in the package insert. The lower limit of detection for that assay was 0.54 ng/mL.

Measurement of fibrinogen

Fibrinogen levels in BAL samples were determined with ELISA, using a 1 : 5000 dilution of sheep antihuman fibrinogen antibody (The Binding Site Ltd, Birmingham, England) as the primary antibody and a 1 : 3000 dilution of horseradish peroxidase-conjugated sheep antihuman fibrinogen (Dako Corp., Carpinteria, CA) as the secondary antibody. The assay curve for the fibrinogen standard (Calbiochem, La Jolla, CA) ranged from 2 to 512 ng/mL [18].

Statistical analyses

BAL ragweed-specific IgE, IgA, sIgA and IgG were considered primary outcome variables in this study. Because many samples had undetectable levels of these antibodies, in the assessment of the relationship between these antibodies and the outcomes of the late allergic reaction, we chose to follow a two-group unpaired analysis model: the two groups consisted of (a) those subjects with detectable levels at baseline and (b) those with undetectable levels. Comparisons between the two groups were performed either with unpaired *t*-test (when the dependent variable was normally distributed, such as in the case of FEV₁ or antigen log PD₂₀), or with the Mann Whitney *U*-test (when the dependent variables were not normally distributed as in the case of the allergen-induced changes in BAL fibrinogen and ECP). For the same reasons, a similar, two-group analysis was undertaken when serum ragweed specific IgG was assessed. To confirm the findings of the dichotomous approach, we also performed non-parametric correlations using the Spearman rank correlation coefficient (r_s); in this analysis, the immunoglobulin levels were treated as a continuous variable; samples with levels below the lower limit of detection were assigned a value that was half the limit of detection of the assay. In every case, this analysis confirmed the results of the dichotomous variable approach. In the case of serum IgE and IgA levels, due to the fact that almost all subjects had detectable levels, a continuous variable approach was used as the primary analysis and parametric statistics were employed with logarithmic transformation which yielded normally distributed values.

In secondary analyses, paired *t*-tests or the Wilcoxon matched-pairs test (depending on the distribution of the

variable in question) were used to compare visits 1 and 3 with respect to various lung function and BAL or serum cellular and biochemical outcomes. Simple regression coefficients (*R*) were calculated to assess the relationships between various outcomes of the allergen-induced changes in lung function. The Spearman rank correlation coefficient was used to examine relationships among BAL immunoglobulin isotypes.

Two-tailed *P*-values ≤ 0.05 were considered significant. All computations were performed using Statview 5.0 software (SAS Institute Inc., Cary, NC). All data in the text, tables and figures are presented with mean $\pm$ SEM values.

Results

Lung function outcomes

Per design, all subjects had at least a 20% acute reduction in FEV₁ following the inhalation challenge with aerosolized ragweed, on visit 2. Between the 4th and 8th hour after the end of the allergen challenge, the lowest FEV₁ value (the nadir) expressed as a percentage of the value obtained following the diluent challenge, ranged from 43% to 105%. Six subjects had FEV₁ values that were less than 85% which, according to convention, are considered late phase reactions. No significant relationship between the antigen log PD₂₀ and the nadir FEV₁ between the 4th and 8th hours after antigen challenge was found ($R = 0.07$; $P = 0.80$), indicating that the size of the late phase reaction was unrelated to the amount of ragweed inhaled during the challenge. Consistent with our previous observations [19] and with those of other investigators [20,21], there was no relationship between the FEV₁ at the conclusion of the antigen challenge vs. the FEV₁ at the nadir of the late phase reaction ($R = 0.1$; $P = 0.70$), showing that the magnitude of the late phase reaction is not dependent on that of the immediate response.

Relationships between immunoglobulin levels and the ragweed-induced reaction

The primary aim of this study was to assess the relationship between ragweed-specific IgE in BAL fluids and the physiological and inflammatory manifestations of the late allergic reaction induced by ragweed inhalation challenge. To put this relationship in perspective, we compared it with the relationships between the late reaction and other immunoglobulin isotypes in both the BAL fluid and in serum.

The analysis we performed was based on separating our subjects into two groups: those with and those without detectable levels of a particular isotype in baseline (visit 1)

Table 1. Concentrations of ragweed (Rgw) specific antibodies measured in baseline (visit 1) serum and BAL fluids

	Subjects with detectable levels	Mean $\pm$ SEM (ng/mL)	Range (ng/mL)
Rgw specific IgE			
Serum	13/16	88.3 $\pm$ 46.1	1.25–731.7
BAL	4/16	1.2 $\pm$ 0.9	0.03–12.1
Rgw specific IgA			
Serum	16/16	54.1 $\pm$ 16.4	8.6–257.7
BAL	5/16	9.2 $\pm$ 7.5	0.5–120.1
Rgw specific sIgA			
BAL	6/16	8.2 $\pm$ 6.9	0.4–111.5
Rgw specific IgG			
Serum	6/16	380.1 $\pm$ 202.4	60–3322
BAL	1/16	10.9 $\pm$ 1.9	25–39

The BAL fluids were concentrated ten-fold before assaying. In order to present immunoglobulin values in unconcentrated BAL fluids, the values in this table resulted by dividing the immunoassays' outcomes by 10. To calculate means and SEMs, individual data below the limit of detection were assigned a value that was half the lowest limit of detection for that assay.

BAL fluids. This analysis was deemed more appropriate for illustrative purposes, compared to a correlation approach, due to the fact that only a minority of subjects had detectable IgE levels in their BAL fluids (Table 1). Nevertheless, Spearman rank correlation coefficients were also calculated yielding, in every instance, identical results to the unpaired, two-group analysis.

In Fig. 1, the average nadir FEV₁ between hours 4 and 8 is plotted for the aforementioned two groups. The four subjects with detectable ragweed-specific IgE levels in visit 1 BAL fluid had strikingly lower lung function (approximately 30% difference in nadir FEV₁), compared to the remaining 12, in whom IgE was too low to be detected by our assay. In fact, three out of the four subjects with detectable ragweed-specific IgE fulfilled the conventional criterion for a late phase reaction, while the fourth subject missed the criterion by 1%. In contrast, only three out of the 12 subjects with undetectable ragweed-specific IgE in BAL fluid met the late phase criterion. Notably, the two groups were not different with respect to baseline lung function (88.9 $\pm$ 5.5 vs. 85.3 $\pm$ 3.9% predicted, $P = 0.64$) or to baseline airway responsiveness to methacholine (log PD₂₀: 0.96 $\pm$ 0.43 vs. 0.42 $\pm$ 0.16, $P = 0.17$). We also compared the two groups with respect to the area under the time course curve (4–8 h) of FEV₁, which is another measure of the magnitude of the pulmonary late phase reaction. The results of this

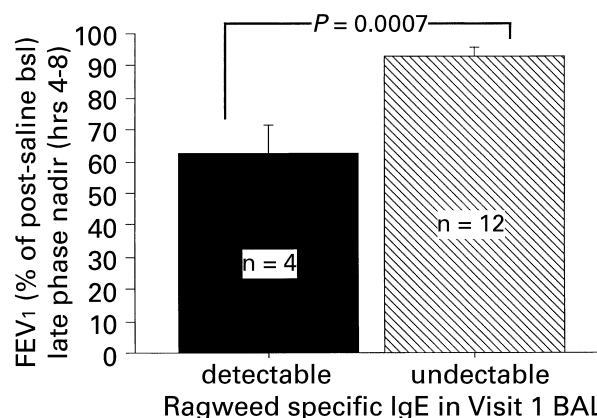


Fig. 1. The presence of antigen-specific IgE in bronchoalveolar lavage (BAL) fluids, during the pre-challenge evaluation (visit 1), is strongly associated with the development of late phase reactions. The mean $\pm$ SEM of the lowest (nadir) FEV₁ measurement obtained between hours 4 and 8 after the end of the ragweed inhalation challenge is presented on the Y-axis as a percentage of baseline (post-saline inhalation). Subjects are stratified into two groups, based on detectability of ragweed-specific IgE in BAL, which is determined by the sensitivity of the assay.

analysis were identical to those from the analysis in which the nadir of the FEV₁ was utilized ($P = 0.0006$ for the difference in area under curve between the subjects with detectable and those with undetectable BAL ragweed-specific IgE).

Figure 2 presents the same type of two-group analysis for the allergen-induced changes in BAL fluid fibrinogen (Panel A) and ECP (Panel B) levels. From these two markers it becomes apparent that the subjects who had detectable levels of ragweed-specific IgE in BAL fluids, at baseline, had the strongest humoral evidence of inflammation (plasma extravasation and eosinophil activation) 24–26 h after allergen inhalation challenge. It is worth noting that allergen-induced increases in BAL fibrinogen were seen in all four subjects with detectable ragweed-specific IgE. In contrast, in 10 out of the 12 subjects from the group with undetectable ragweed-specific IgE, fibrinogen levels in BAL decreased after allergen challenge. Comparison of the two subject groups with respect to the allergen-induced changes in BAL cytology did not yield any significant differences despite the fact that, in the entire group of asthmatics that participated in this study, significant increases in the total number of eosinophils and neutrophils in BAL fluids from visit 1 to visit 3 were recorded (Table 2).

The levels of ragweed-specific IgE in the serum were also related to various aspects of the late phase reaction. Figure 3 illustrates a simple regression of the nadir FEV₁ between hours 4 and 8 after ragweed inhalation challenge

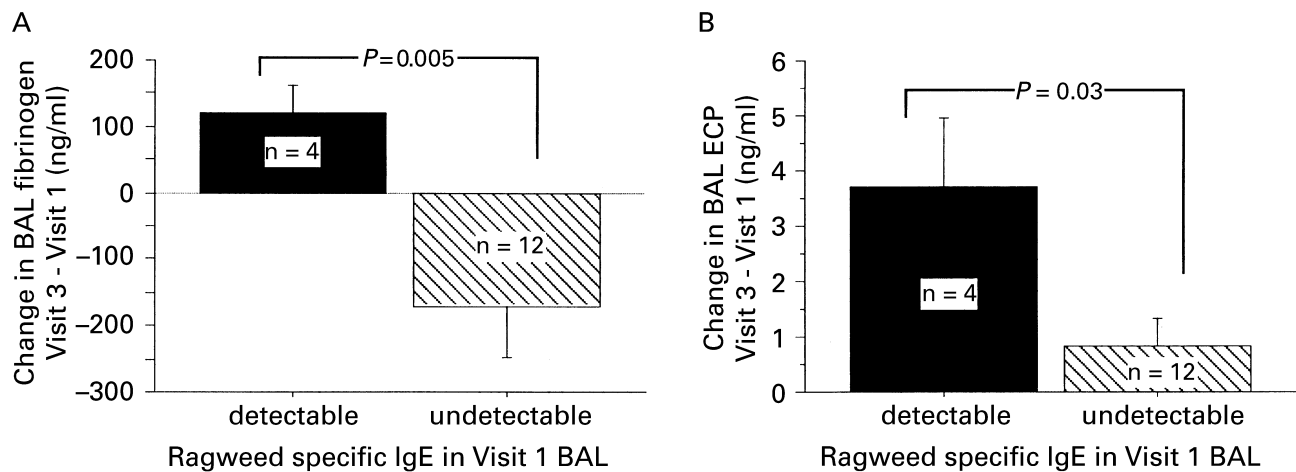


Fig. 2. The presence of antigen-specific IgE in bronchoalveolar lavage (BAL) fluids, during the pre-challenge evaluation (visit 1), is strongly associated (A) with plasma extravasation (assessed by the change in the levels of fibrinogen in BAL) and (B) with eosinophil activation (assessed by the change in the levels of ECP in BAL) 24 h after whole lung ragweed inhalation challenge. Bars represent mean $\pm$ SEM values of the differences between the pre-challenge evaluation (visit 1) and the 24 h post-allergen challenge (visit 3) BAL measurements of the respective biochemical markers. Subjects are stratified into two groups, based on detectability of ragweed-specific IgE in BAL, which is determined by the sensitivity of the assay.

against the log of baseline (visit 1) ragweed-specific serum IgE ($R = 0.72$, $P = 0.002$). Significant correlations between baseline ragweed-specific serum IgE and the allergen-induced change in BAL ECP levels, as well as the change in BAL neutrophils, were also detected ($R = 0.50$, $P = 0.05$ in both cases).

Visit 1 BAL-ragweed specific IgA, when analysed on the basis of a comparison between subjects with detectable vs. undetectable levels, yielded a statistically significant relationship to the physiological but not to the cellular or humoral aspects of the allergen-induced late reaction. The two groups were different in terms of the 4–8 h FEV₁ nadir values expressed as percentage of the post-saline challenge (70.3 ± 10.6 vs. 92.4 ± 3.2 , $P = 0.02$). Analysed in a similar fashion, baseline BAL ragweed-specific sIgA and IgG failed to demonstrate any significant relationships to any aspect of the late allergic reaction (data not shown). Baseline serum ragweed-specific IgA also correlated with the magnitude of the late phase airflow limitation ($R = 0.5$,

$P = 0.05$) but with no other index of the late allergic reaction. Serum IgG had no relationship to any parameters we tested.

There were significant relationships between the serum and BAL fluid levels of ragweed IgE and IgA: in the case of IgE, subjects with detectable levels in BAL on visit 1 had at least 10 times higher serum allergen-specific immunoglobulin, compared with subjects with undetectable BAL levels (log of serum ragweed-specific IgE in ng/mL: 2.2 ± 0.3 vs. 0.9 ± 0.2 , $P = 0.005$); for ragweed-specific IgA, subjects with detectable BAL levels had approximately four times higher serum antibodies compared to the subjects with undetectable BAL IgA levels (log of serum ragweed-specific IgA in ng/mL: 1.9 ± 0.2 vs. 1.3 ± 0.1 , $P = 0.005$). We also detected a relationship between ragweed-specific IgE and IgA levels in BAL fluids: in 13 out of the 16 subjects the two isotypes were concordant in terms of detectability and a quantitative relationship was present ($r_s = 0.67$, $P = 0.009$).

Table 2 Mean number ($\pm$ SEM) of cells ($\times 10^{-6}$) in the bronchoalveolar lavage fluid on visits 1 and 3

	Total cells	Macrophages	Lymphocytes	Neutrophils	Eosinophils	Bronchial cells
Visit 1	13.08 ± 2.05	10.77 ± 1.99	1.69 ± 0.43	0.32 ± 0.09	0.25 ± 0.07	0.06 ± 0.02
Visit 3	15.92 ± 1.44	12.08 ± 1.19	1.94 ± 0.25	$0.95 \pm 0.44^*$	$0.90 \pm 0.43^\dagger$	0.05 ± 0.02

*Difference between visits 1 and 3, $P = 0.007$.

† Difference between visits 1 and 3, $P = 0.003$.

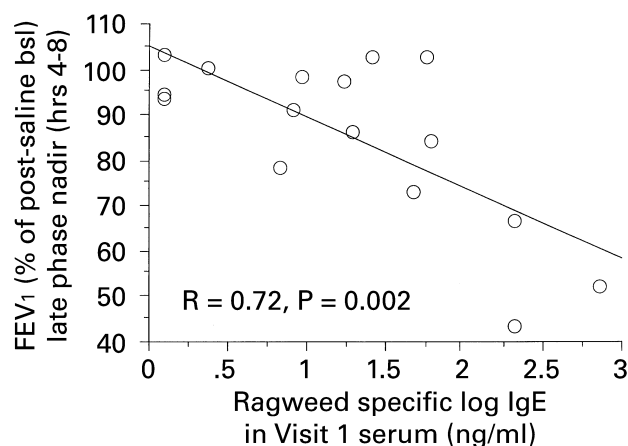


Fig. 3. Antigen specific IgE in serum, during the pre-challenge evaluation (visit 1), is strongly associated with the development of late phase reactions. The values on the Y-axis of the scattergram are the lowest (nadir) FEV₁ measurements obtained from each subject ($n = 16$) between hours 4 and 8 after the end of the ragweed inhalation challenge. These are expressed as percentages of baseline (post-saline inhalation). Serum ragweed specific IgE has been logarithmically transformed.

To further place the relationships of ragweed specific IgE and IgA and the late phase reaction in perspective, we examined whether any relationships between the various immunoglobulin isotypes and the log of antigen PD₂₀ obtained from visit 2, which can be regarded an index of the acute airway response to antigen challenge, existed. This entire analysis yielded negative results (data not shown).

Discussion

The main finding of this study is that baseline levels of ragweed-specific IgE and IgA in BAL fluid correlate with the magnitude of the airway response that occurs during the late phase of an allergic reaction. For ragweed-specific IgE, this relationship is also observed between the baseline immunoglobulin levels in BAL and allergen-induced inflammatory changes including plasma extravasation (reflected in the levels of fibrinogen) and eosinophil activation (reflected in the levels of ECP). These findings fully confirm our previous work with a bronchoscopic, subsegmental allergen challenge model in which a relationship between ragweed-specific IgE and IgA in BAL fluids and the allergen-induced change in ECP levels was established [10] and extend these observations to airflow limitation, the physiological outcome of the late phase reaction induced by whole lung antigen provocation.

The association between IgE and the allergic late phase reaction was first demonstrated by the seminal studies of

Dolovich and Solley [22,23], using the skin as the *in vivo* model. In these studies, however, the early and the late phase reactions were not dissociated, that is, these investigators did not attempt to address the question whether the relationship between IgE and the late phase was an epiphenomenon of the relationship between IgE and the early phase. Also, their work was in reference to total, rather than specific, IgE. In 1974, Robertson and colleagues, in a group of 15 ragweed-allergic asthmatics, found measurable levels of serum antigen E-specific IgE only in the eight subjects who developed a late phase reaction following bronchial challenge with ragweed [24]. This work was confirmed by Atkins and colleagues who showed that the concentration of antigen-specific IgE in serum was associated with the degree of allergen-induced late phase inflammation in the human skin [25]. Durham and colleagues, on the other hand, did not report similar results [26], but their methodology for measuring serum specific IgE was semiquantitative.

Our findings further the concept of the relationship between IgE and the allergic late phase reaction by extending the original observations to antigen-specific antibodies in BAL fluid. What makes this report even more important is that the association we have detected seems to be independent of any association between antigen-specific IgE and the early response to allergen; such an association was absent in our study. Cockcroft and colleagues have previously reported significant associations between skin prick test reactivity to allergenic extracts and the concentration of allergen required to induce acute airflow limitation [27,28] but these associations have been disputed by others [29,30]. The independence of the association between antigen-specific IgE and the late phase from that between antigen-specific IgE and the early allergic reaction that we are reporting supports the notion that antigen-specific IgE plays an active role in the late phase. However, on the basis of a mere association such a role cannot be proven. Also, our work does not offer any clues as to the mechanism through which antigen-specific IgE may play a role in the late phase reaction.

We have recently reported that the pulmonary late phase reaction is almost fully reversible by an intravenous beta-agonist, suggesting that smooth muscle contraction is the main mechanism of airway obstruction [14]. Given that elevated levels of spasmogenic autacoids are found in serum [26] and in BAL fluids during the late phase [31,32], it is reasonable to postulate that these are responsible for the smooth muscle spasm. In fact, a recent study using a combination of an antihistamine and a leukotriene receptor antagonist showed remarkable inhibition of the late phase reaction [33]. If autacoid release is central in the generation of airflow limitation during the late phase allergic reaction one could develop a hypothesis regarding the role of IgE in

this process. It is possible that allergen remaining in the airways after the end of the provocation activates inflammatory cells which migrate to the site a few hours after the acute allergic reaction. Basophils are primary candidates in this scenario. These cells are known to accumulate at the site of allergic reactions [34,35] and to produce spasmogens that are present in late phase BAL fluids [31]. Such activation of basophils would occur through the interaction of allergen with plasma membrane-bound specific IgE and it would not be surprising if the number of IgE molecules attached to Fc ϵ RI receptors on the surface of these cells was in equilibrium with serum, tissue and BAL free IgE.

Recently, IgE receptors have been identified on other inflammatory cells, including eosinophils [1–3] and monocytes/macrophages [4,5], which also participate in allergic reactions and also produce spasmogens, especially sulfidopeptide leukotrienes [36,37]. Eosinophils are of particular interest in late phase pathogenesis given that the granules of these cells have cytotoxic effects of airway epithelium and suppressive effects on the inhibitory muscarinic M₂ receptor [38]. The surface expression of Fc ϵ RI has recently been reported to be upregulated in the presence of IL-4 and IL-13 [39], cytokines previously shown to be produced in antigen challenge models such as ours. Even some of the eosinophil basic proteins can contract human airway smooth muscle [40].

An alternative explanation for the release of autacoids during the late phase reaction through a mechanism involving IgE would invoke the well-described and cloned IgE-dependent histamine releasing factor [41,42]. This cytokine has been associated in the past with the nasal late phase [43], while other histamine releasing factors have been found in skin blister fluids during cutaneous late phases [44]. However, IgE-dependent autacoid releasing factors would not require allergen-specific IgE to activate cells and we did not measure total IgE in the BAL fluids, to examine whether it also correlates with the late phase reaction; thus, we cannot provide further insight into this matter.

The finding of only four subjects with detectable levels of ragweed-specific IgE in baseline BAL fluids is probably reflective of the sensitivity of the detection methodology. Robertson and colleagues [24], in their study relating serum antigen specific IgE and the late phase reaction, 'assumed that IgE antibodies to ragweed antigen E were present in the sera of all these ragweed allergic subjects with concentrations too low for detection in the subjects with isolated early asthmatic responses'. A similar situation was probably encountered in our subjects' BAL fluids. The possibility exists that the relatively low levels of ragweed-specific immunoglobulins in BAL reflect proteolytic cleavage of these proteins by enzymes present in the

respiratory fluids. This possibility, for which no good evidence exists in humans, should always raise caution as to the interpretation of any form of protein data obtained from BAL. However, the validity of the BAL measurements is supported by the fact that they reflected the measurements of ragweed-specific IgE in serum. Hence, the separation of our subjects into two groups on the basis of assay limitation is very reasonable. The difference in the magnitude of the late reduction in lung function between the four subjects with detectable antigen specific IgE in baseline BAL fluids and those with undetectable levels was so profound that, even with this small number of subjects in one group, the results were highly statistically significant. This finding should negate any potential concerns regarding the small number of subjects with detectable ragweed-specific IgE in BAL fluids.

The source of antigen-specific IgE in BAL fluids cannot be determined from this study. The strong association between the BAL and the serum concentrations suggests that BAL IgE reflects a state of equilibrium between circulating and tissue immunoglobulin. This state of equilibrium, however, should not be regarded as a simple 'spill over' effect. In further analysis of our data, we found that there was no relationship between fibrinogen levels and ragweed-specific IgE in BAL fluid on visit 1 (data not shown), suggesting that the ragweed-specific IgE in the airway fluid is not solely due to plasma leakage. In addition, we did not find a relationship between the BAL levels of fibrinogen on visit 1 and the magnitude of the late phase reaction. Therefore, the relationship of ragweed-specific IgE and the late phase reaction is not an epiphenomenon of pre-existing inflammation and vascular leakage. The tissue and, consequently, BAL levels of the antibody may be more important in the biological effects of this molecule, when local (lower airway) reactions such as the one we have induced are considered. With this notion in mind, our efforts have been concentrated on the BAL compartment for quantitating these immunoglobulins and for attempting to assess their potential role in the late phase reaction.

The association between ragweed-specific IgA and the late phase reaction is intriguing. The data we have obtained confirm our previous findings with subsegmental allergen challenge model [10]. Whether our finding reflects an epiphenomenon (resulting from the strong association between BAL ragweed-specific IgE and IgA) or whether it is indicative of an active role of IgA in the late phase reaction is a matter that will require further evaluation. The theoretical basis for the latter possibility, however, exists: eosinophils carry receptors for IgA (Fc α R) which can mediate cell degranulation [6,7].

In summary, this study is the first to report a relationship between local, antigen-specific IgE antibodies and the

physiological and biochemical aspects of the late phase reaction in the lower airway of patients with asthma. The mechanism behind this relationship is unknown. This observation may lead to studies that will shed light into the pathophysiology of the late phase reaction and its relationship to allergic airway disease.

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