

- muscle from patients with chronic airflow obstruction. *Thorax* 1989;44:28-35.
33. Rogers AW. Techniques of autoradiography. Amsterdam: Elsevier Scientific, 1973.
 34. Vecerina S, Krajina Z, Krmpotic J, Zirdum A. Comparative analysis of human nasal mucosa by standard neurohistochemical techniques. *Rhinology* 1983;21:73-8.
 35. Davis B, Marin MG, Yee JW, Nadel JA. Effect of terbutaline on movement of Cl^- and Na^+ across the trachea of the dog in vitro. *Am Rev Respir Dis* 1979;120:547-52.
 36. Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic subtypes in human lung. *Am Rev Respir Dis* 1985;132:541-7.
 37. Welsh MJ. Electrolyte transport by airway epithelia. *Physiol Rev* 1987;67:1143-9.
 38. Hall LJ, Jackson RT. Effects of alpha- and beta-adrenergic agonists on nasal blood flow. *Ann Otol* 1968;77:1120-30.
 39. Malm L. Sympathetic influence on the nasal mucosa. *Acta Otolaryngol* 1977;83:20-1.
 40. Kaliner M, Austen KF. Immunologic release of chemical mediators from human tissues. *Ann Rev Pharmacol* 1975;15:177-89.

Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: Relationship to the development of late asthmatic responses

Sally E. Wenzel, MD,* Jay Y. Westcott, PhD,** and Gary L. Larsen, MD*
Denver, Colo.

Inflammatory mediators have been implicated in the pathogenesis of human asthma and have been demonstrated to increase in bronchoalveolar lavage fluid during the time of the immediate asthmatic response (IAR) after allergen instillation in the lungs. However, the relationship of these mediators, measured early to the late asthmatic response (LAR), airway reactivity, and clinical asthma, is unknown. In the present study, we evaluated mediator levels in bronchoalveolar lavage fluid before and 5 minutes after allergen challenge from three subject groups: atopic subjects without asthma (N = 7), atopic subjects with asthma and without LAR ((-) LAR) (N = 6), and atopic subjects with asthma and with LAR ((+) LAR) (N = 6). Subjects with asthma were differentiated into subjects with and without LARs based on at least a 15% decrease in FEV₁ between 3 to 8 hours postallergen inhalation. The mediators, prostaglandin D₂, thromboxane B₂, leukotriene C₄ (LTC₄), and histamine, were measured both before and after allergen instillation. Baseline prechallenge levels were similar, except in the case of LTC₄. LTC₄ was detectable at baseline significantly more frequently in the atopic subjects with asthma with and without LAR when these subjects were compared to the atopic subjects without asthma (nine of 12 detectable versus one of seven detectable). In all groups, significant increases in mediator levels were observed in the groups with asthma postallergen challenge, compared to the atopic subjects without asthma. Atopic subjects with asthma and without LAR had significantly higher levels of all four mediators after challenge than atopic subjects with asthma and with LAR and atopic subjects without asthma. No correlation was found between the mediator levels and airway physiology among any of the three groups. Although atopic subjects with asthma and with LAR were more reactive to methacholine than the other two groups, the difference did not reach significance. In addition, no correlation was observed with cell counts, differentials, or proteins. It is hypothesized from these findings that multiple factors are involved in the production of an LAR. These factors most likely include mediator levels and underlying airway reactivity, with the more reactive airways requiring less mediators for bronchoconstriction to occur. This finding implies that therapeutic options in asthma should focus not only on blockage of mediator activity but also on reversal of underlying airway hyperreactivity. (J ALLERGY CLIN IMMUNOL 1991;87:540-8.)

From the *Departments of Medicine and Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo., and **Webb-Waring Lung Institute and Cardiovascular Pulmonary Research Department, University of Colorado School of Medicine, Denver, Colo.

Supported by National Institutes of Health Grants NRSA HL-07433, HL-36577, and HL-34303.

Received for publication June 19, 1990.

Revised Oct. 2, 1990.

Accepted for publication Oct. 2, 1990.

Reprint requests: Sally E. Wenzel, MD, Assistant Professor of Medicine, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206.

1/1/25934

Abbreviations used

LAR:	Late asthmatic response
IAR:	Immediate asthmatic response
BALF:	Bronchoalveolar lavage fluid
PGD ₂ :	Prostaglandin D ₂
Tx:	Thromboxane
LTC ₄ , LTE ₄ , LTD ₄ :	Leukotrienes C ₄ , E ₄ , D ₄
PD ₂₀ :	Provocative dose causing 20% fall in FEV ₁

A variety of mediators have been implicated in the pathogenesis of human asthma, but their role in the production of the antigen-induced LAR is unknown. In contrast, the contribution of mediators to the IAR has been suggested by the presence of substances such as histamine and PGD₂ in both BALF and serum.¹⁻⁴ In addition, both inhibitors and antagonists of a variety of mediators present during the time of the IAR are known to partially or completely block the IAR.^{5, 6} However, the relevance and/or necessity of the events that constitute an IAR to the eventual production of an LAR is controversial. Although it is possible that mediators released during the IAR either directly through continuous low level production or indirectly through attraction and/or stimulation of other cells induce the LAR, evidence to support this supposition is lacking. It has recently been suggested that, in sheep, the amount of sulfidopeptide leukotrienes generated during the IAR may determine whether an LAR develops.⁷ However, direct measurement of leukotrienes during the IAR was not performed. In contrast, some investigators would argue that, when the IAR is abolished physiologically by antihistamines or β -agonists, the LAR will still occur or be accentuated because of the larger antigen dose that can be administered in the presence of these agents.⁸ This finding suggests, at least, that a physiologic response is not necessary during the IAR for an LAR to occur. However, these studies can not definitely conclude whether the mediators produced during the IAR affect the LAR. Although the effects of these drugs may block the physiologic IAR, other cellular events, including mediator release, may still occur during that time, leading to the LAR.

We have previously measured mediator levels in BALF in atopic subjects with asthma and have demonstrated large increases in histamine, PGD₂, TX, and LTC₄ after allergen challenge. The mediator levels in BALF from atopic subjects with asthma were higher after allergen challenge than levels in control subjects that included nonatopic subjects with asthma, atopic subjects without asthma, and normal subjects.^{2, 3, 9} It was apparent from these studies that significant variability existed in the subjects with asthma regarding the mediator levels, although the reason for this variability was not known. We speculated that a relation-

ship existed between the amount of mediators released during the time of the IAR and the subsequent production of an LAR. Whether this relationship was further influenced by the underlying airway reactivity, the dose of allergen administered, or the amount of physiologic response during the IAR was unknown.

To test this hypothesis, we recruited atopic subjects with asthma and divided the subjects into two groups based on the presence or absence of an LAR after inhaled allergen challenge. After a 2 to 4-week recovery period, these subjects, as well as a control group of atopic subjects without asthma, underwent a bronchoscopy with local instillation of allergen. The subjects were lavaged before and 5 minutes after the challenge. The lavage fluid was analyzed for cell counts, protein, histamine, and the eicosanoids, PGD₂, TX, and LTC₄. Mediator levels were then compared with the amount of allergen administered, airway reactivity measured before allergen challenge, and the presence or absence of an LAR. The data demonstrate that all subjects with asthma have an increase in mediators after challenge compared to the atopic control subjects without asthma but that subjects without an LAR have significantly greater amounts of mediators in BALF 5 minutes after antigen challenge than those subjects with LAR.

MATERIAL AND METHODS

Patient selection

Three groups of volunteer nonsmoking subjects, between 18 and 50 years of age, were recruited for study. The groups included atopic subjects without asthma, atopic subjects with asthma and without LAR, and atopic subjects with asthma and with LAR. Testing was done on 2 or 3 separate days, during a period of 2 to 4 weeks. Day 1 consisted of the initial clinical screening, allergen skin testing, and methacholine challenge. This testing procedure was done to determine clinical allergy and asthma history, allergen sensitivity, and airway reactivity. Day 2 consisted of an allergen-inhalation challenge, whereas day 3 consisted of bronchoalveolar lavage before and after local allergen instillation. Testing on day 2 was not performed in atopic subjects without asthma because of concerns of the Institutional Review Board regarding the long-term effects of allergen-inhalation testing on subjects without asthma. In this study, clinical asthma was defined as a history of documented wheezing and/or cough responsive to bronchodilators. A clinical history of specific precipitating allergens included symptoms of wheezing or rhinitis seasonally to weeds (fall), grasses (spring), trees (early spring), or the production of the same symptoms by cats or molds for at least 3 years. Skin testing was performed with diluent and histamine controls in parallel with a battery of 10 allergens common in Colorado. Testing was done by skin prick method at a 1:10 (wt/vol) dilution of a variety of stock solutions (Greer Laboratories, Lenoir, N.C.). A positive response was considered to be a wheal of 5 by 5 mm.¹⁰ Any one positive response determined the subject to be atopic.

Spirometry (Eagle II, Warren E. Collins, Inc., Braintree,

TABLE I. Subject data

	Age*	Methacholine† PD ₂₀ (mg/ml)	FEV ₁ /FVC*	Allergen* wheal diameter (mm)
Atopic				
Without asthma	31 ± 4	1 = 4.3 6 of 7 > 8.0	90.4 ± 1.8	12.1 ± 1.4
Asthma (-LAR)	28 ± 3	0.23 (0.03 - 1.47)‡	72.0 ± 5.5‡	11.3 ± 1.6
Asthma (+LAR)	35 ± 4	0.09 (0.03 - 0.3)‡	65.8 ± 4.8‡	10.7 ± 1.9

*Mean ± SEM.

†Geometric mean with range.

‡*p* < 0.05 compared to atopic subjects without asthma.

Mass.) was performed before methacholine challenge. If the FEV₁ was >60% of predicted, the subject proceeded to the methacholine challenge. The methacholine challenge consisted of the inhalation of doubling concentrations of methacholine (from 0.3 to 16 mg/ml) from a DeVilbiss nebulizer (DeVilbiss Corp., Somerset, Pa.) until the FEV₁ decreased by 20% or more, as previously described.¹¹ Five vital capacity breaths with inhalation of diluent or methacholine were followed in 5 minutes by measurement of the FEV₁. When the FEV₁ fell by 20% or greater, or the maximal dose was reached, testing was stopped, and the (PD₂₀) was determined by extrapolation from the dose-response curve (Table I).

Nineteen subjects were entered into the study at this point. Twelve subjects met criteria for asthma and proceeded to day 2 of the study. Seven subjects served as atopic control subjects with no clinical history of asthma or increased airway reactivity. As previously mentioned, these subjects did not undergo day 2 testing. The 12 subjects with asthma underwent allergen-inhalation challenge as follows: Theophylline preparations were held for 24 hours before study, whereas β-agonists were stopped 8 hours before challenge. No subject was taking inhaled or oral steroids or cromolyn. All subjects began the study between 7 and 7:30 AM. The subjects inhaled logarithmically increasing concentrations of allergen beginning at 1:1,000,000 and proceeding to 1:100 (wt/vol). If the FEV₁ did not fall by 20% or greater at 1:100, that dose was repeated. At each point, the patient inhaled 10 tidal volume breaths from a Hudson (Hudson Respiratory Care, Inc., Temecula, Calif.) nebulizer. The FEV₁ was measured at 5-minute intervals until the maximum drop was measured (as evidenced by an increase in the FEV₁ of 5% or more from the previous level). If the FEV₁ had not fallen by 20% after 15 minutes, the next concentration was administered.

Once a 20% drop in FEV₁ was measured, spirometry was performed at 15-minute intervals for the first hour and then hourly for the next 12 hours. An LAR was defined as a 15% or greater fall in FEV₁ from the prechallenge baseline occurring between 3 to 12 hours after challenge.

Subjects were excluded from the study if they were pregnant, had a history of heart disease, were currently receiving steroids of any kind, or were receiving allergen immuno-

therapy. All subjects were tested out of the respective allergen season or after attempts to minimize environmental (i.e., cat) exposure. All subjects gave informed consent, and the study was approved by the National Jewish Institutional Review Board.

Bronchoalveolar challenge and lavage

At least 2 weeks after allergen-inhalation and methacholine challenge, subjects returned to the hospital for bronchoalveolar lavage. Respiratory medications were withheld as for the allergen-inhalation challenge. If the FEV₁ varied <10% from baseline, bronchoscopy was performed following previously described guidelines.¹² Premedication consisted of intramuscular atropine (0.6 mg) and codeine (30 mg). Intravenous access, oxygen therapy, and oximetry monitoring were maintained throughout the procedure. Just before bronchoscopy, subjects received 1 to 2 mg of intravenous midazolam. Once again, all studies were begun between 7 and 7:30 AM. Airway anesthesia consisted of lidocaine (4%) aerosolized to the upper airways, posterior pharynx, and epiglottis. The bronchoscope (Olympus BR 4B; Olympus Corp. of America, New Hyde Park, N.Y.) was passed nasally, and lidocaine (2%) was delivered directly onto the vocal cords before passage into the lungs. Once in the lungs, 1 to 2 ml of lidocaine (2%) was used at the carina and again just before the wedge. After wedging in a fourth to fifth order bronchus of the lingula, four aliquots (37 ml) of sterile saline at 37° C were instilled through the bronchoscope and sequentially removed under gentle manual aspiration. The bronchoscope was then repositioned in a corresponding subsegment of the right lung. Five milliliters of allergen to which the subjects demonstrated skin sensitivity were instilled at varying concentrations, depending on allergen-inhalation challenge (in the subjects with asthma) or skin testing (in the subjects without asthma). The subjects with asthma received 5 ml of a tenfold lower concentration than that which caused a 20% decrease in the FEV₁ on inhalational challenge with five subjects without LAR receiving 1:1000 and one receiving 1:10,000; three subjects with LAR receiving 1:1000, one receiving 1:10,000 and two subjects receiving 1:100,000. The atopic subjects without asthma received allergen based on skin testing data. A concentration of 1:10,000 was administered

to subjects with wheals >15 mm and 1:1000 was administered for wheals 10 to 15 mm. In the group studied, five received 1:1000 and two subjects received 1:10,000. Subjects with asthma and without LAR received ragweed ($N = 1$) or cat allergen ($N = 5$), whereas subjects with asthma and LAR were administered timothy grass ($N = 2$), *Alternaria* ($N = 1$), and cat allergen ($N = 3$). Atopic subjects without asthma received either ragweed ($N = 3$) or timothy grass allergen ($N = 4$). Mean wheal diameters with range, as an indication of allergen sensitivity, are listed in Table I. All measurements were obtained at a 1:10 dilution with the skin prick method. Antigens were distributed as noted for the bronchoalveolar challenge. Although the amount of antigen administered to the atopic subjects without asthma is somewhat arbitrary, in view of the fact that allergen inhalational studies were not performed, we believed it was the best indication of allergen sensitivity that we had available. The amount administered was very similar to, if not higher than, that administered to the two groups with asthma.

Processing of BALF

The four BALF aliquots collected before and after allergen challenge were combined immediately after the procedure. The pre- and postallergen lavage samples were then centrifuged at 4°C to separate fluid from cells. A portion of each supernatant was aliquoted into 2 vol of iced methanol and frozen at -70°C . The remainder was stored at -70°C for analysis of protein and histamine. Cell counts and viability testing were performed with a hemacytometer and trypan blue exclusion staining, with results expressed as viable cells per milliliter of lavage fluid. Differentials were done with a Diff-Quik stain (American Scientific Products, McGraw Park, Ill.) and counting 200 cells.

Analytical methods

Protein was analyzed with a modified Lowry technique.¹³ Histamine was assayed by a modification of the single isotope radioenzymatic technique previously described,^{14, 15} which is sensitive to 100 pg/ml. This method used histamine *N*-methyltransferase prepared from rat kidneys.¹⁵ Intra-assay variation was $<10\%$.

The eicosanoids, PGD_2 , TX, and LTC_4 , were measured with enzyme immunoassays as previously described.¹⁶ [^3H]LTE₄ (15,000 disintegrations per minute; 79 pg, New England Nuclear, Boston, Mass.) was added to each BALF sample as a marker for leukotriene recovery being used instead of [^3H]LTC₄ because of the lower mass added with the [^3H]LTE₄. Eicosanoids were partially purified with octadecylsilyl Sep-Pak (Waters Associates, Milford, Mass.) cartridges. Prostanoids were eluted in 5 ml of 1% methanol/ethyl acetate, whereas leukotrienes were eluted in 5 ml of 80% methanol/water. Previously, recovery of radiolabeled prostanoids was found to be 80% to 92% in a similar set of samples.³ In the present sample, recovery was $>80\%$ for peptidoleukotrienes. Samples were then evaporated under negative pressure and redissolved in a phosphate-buffer for analysis by enzyme immunoassays. Samples for PGD_2 were first derivitized to PGD_2 -methoximine with the pre-

viously described method.¹⁷ The prostanoid assays used rabbit polyclonal antibodies, which were the gifts of Dr. Jacques Maclouf (INSERM, Paris, France), whereas LTC₄ was measured with a mouse monoclonal antibody with 100% cross-reactivity to LTD₄, 55% LTC₄, and 51% LTE₄ (Advanced Magnetics, Boston, Mass.).¹⁸ Enzyme tracers were purchased from Cayman Chemicals (Ann Arbor, Mich.). Prior studies of the peptidoleukotrienes with high-performance liquid chromatography and a second leukotriene antibody, more specific for LTC₄, confirmed that most leukotrienes present were LTC₄.⁹ In this study, leukotrienes measured are expressed as immunoreactive LTC₄. Quantitation of LTC₄ in BALF was corrected for recovery of [^3H]LTE₄ through the Sep-Pak.

Statistical analysis

All results were expressed as mean $\pm$ SEM or geometric mean with range. When the levels of the mediators were below the sensitivity level of the assay, the levels were reported in the text and graphically as "nondetectable." Lavage results were evaluated as "per milliliter" of lavage fluid, as in previous studies.^{2, 3, 9} Statistical analysis was performed with one-way analysis of variance with the Dunnett method for repeated measures.¹⁹ Chi-square analysis was used to compare the two groups (subjects with asthma versus without) when nondetectable levels in one of the two groups made comparisons of the means impractical. This was done by analyzing the proportions of detectable to nondetectable levels in each group. Results were analyzed between groups before and after allergen challenge. Correlation analysis was used to compare physiologic parameters, such as FEV₁ (percent predicted) and methacholine PD₂₀ with mediator levels. Statistical significance was defined at $p < 0.05$, unless it is otherwise noted.

RESULTS

Physiologic parameters

As has been reported by other investigators,²⁰ subjects with LARs tended to be more reactive than subjects without LARs (Table I). However, statistical significance for this parameter was not reached ($p = 0.28$). Significant differences in FEV₁/FVC and FEV₁ (percent of predicted) were observed between the groups with and without asthma ($p < 0.05$) (Table I), but not between subjects with asthma with and without an LAR.

Lavage results

As noted in the METHODS section, the percent volume of return in both groups with asthma was significantly less than that from the atopic group without asthma, both before and after allergen instillation. However, there were no significant differences between the two groups with asthma when groups were compared to each other or when the volumes before allergen challenge are compared to after allergen challenge (Table II).

There were no significant differences among the

TABLE II. Lavage data

	Volume (ml)	Cells/ml ($\times 10^4$)	Protein (mg/ml)
Atopic			
Without asthma			
Before	84 $\pm$ 8	10.5 $\pm$ 5.6	129 $\pm$ 20
After	89 $\pm$ 4	6.1 $\pm$ 1.7	95 $\pm$ 12
Asthma (-LAR)			
Before	56 $\pm$ 11*	9.4 $\pm$ 2.0	130 $\pm$ 25
After	47 $\pm$ 9*	9.6 $\pm$ 4.6	163 $\pm$ 31
Asthma (+LAR)			
Before	56 $\pm$ 5*	10.5 $\pm$ 2.8	214 $\pm$ 89
After	47 $\pm$ 8*	5.7 $\pm$ 1.8	157 $\pm$ 32

All values, mean $\pm$ SEM.* $p < 0.05$ compared to atopic subjects without asthma.

TABLE III. Bronchoalveolar cell differentials preallergen challenge

Group	Total cells ($\times 10^4$)	Macrophages	Neutrophils (as % of total)	Eosinophils	Lymphocytes
Atopic					
Without asthma					
Before	10.5 $\pm$ 5.6	94 $\pm$ 1	1 $\pm$ 1	3 $\pm$ 1	2 $\pm$ 1
After	6.1 $\pm$ 1.7	92 $\pm$ 1	1 $\pm$ 1	2 $\pm$ 2	6 $\pm$ 2
Asthma (-LAR)					
Before	9.4 $\pm$ 2.0	90 $\pm$ 2	2 $\pm$ 1	4 $\pm$ 1	4 $\pm$ 2
After	9.6 $\pm$ 4.6	89 $\pm$ 1	1 $\pm$ 1	3 $\pm$ 1	7 $\pm$ 1
Asthma (+LAR)					
Before	10.5 $\pm$ 2.8	88 $\pm$ 2	2 $\pm$ 1	5 $\pm$ 2	5 $\pm$ 3
After	5.7 $\pm$ 1.8	90 $\pm$ 1	3 $\pm$ 1	3 $\pm$ 1	5 $\pm$ 1

All numbers, mean $\pm$ SEM.

three groups when either total cell counts per milliliter of lavage fluid or protein levels were compared (Table II). Cell differentials were not significantly different between any group or before challenge to after allergen challenge. The pre- and postchallenge differentials are listed in Table III.

Mediator levels

Each of the four mediators was measured both before and after allergen challenge. Atopic subjects with asthma both with and without LARs tended to have higher baseline levels of mediators (except for PGD₂) than atopic subjects without asthma. LTC₄ was often below detection in baseline samples but was detectable in five of six subjects with asthma and without an LAR, four of six subjects with asthma with an LAR, and only in one of seven atopic subjects without

asthma ($p < 0.04$ by chi-square analysis for atopic subjects with asthma versus atopic subjects without asthma). After allergen challenge, BALF collected from atopic subjects with asthma both with and without LAR had significant increases in all four mediators compared to atopic subjects without asthma and preallergen challenge (Fig. 1). When the postallergen challenge levels were compared between the two atopic groups with asthma, in the case of all four mediators, the levels were significantly higher in those subjects without LARs than in those subjects with an LAR.

Correlation analysis

In an effort to correlate physiologic changes with mediator levels, the levels of each mediator were compared both pre- and postallergen challenge with

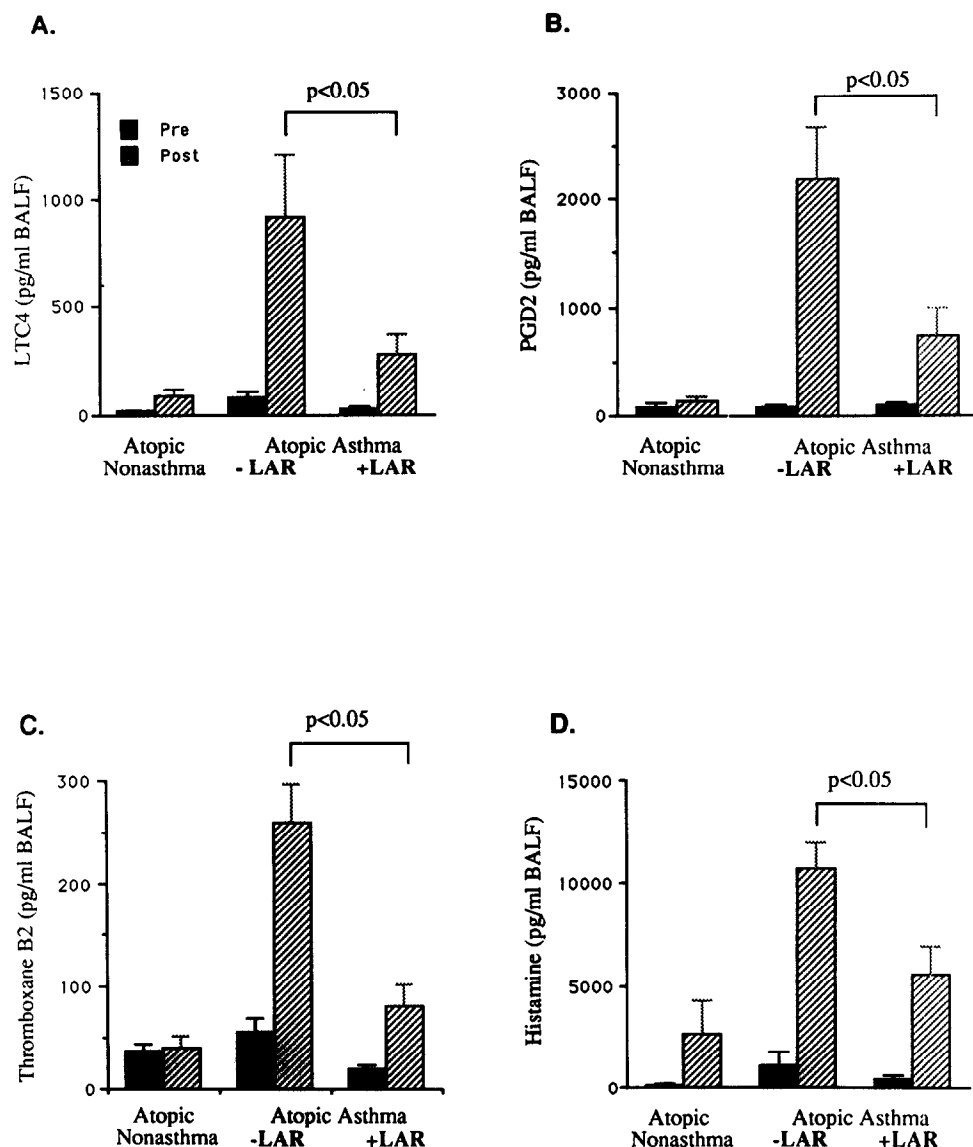


FIG. 1. Concentrations in BALF pre- and postendobronchial allergen challenge from atopic subjects with asthma and with and without LAR, and from atopic control subjects without asthma. **A.** Histamine. **B.** LTC₄. **C.** PGD₂. **D.** TX. Atopic subjects without LAR have significantly elevated levels of all mediators measured compared to atopic subjects without asthma and atopic subjects with asthma and with LAR.

methacholine PD₂₀, FEV₁ (percent of predicted), and FEV₁/FVC. In addition, volume of return and cell counts were compared to these same physiologic parameters.

Preallergen challenge. No significant correlations were observed in preallergen challenge between mediators and physiologic parameters. In contrast, strong correlations were observed between volume of return and prechallenge FEV₁/FVC ($r^2 = 0.476$; $p = 0.0008$), as well as FEV₁ prechallenge (percent of predicted) ($r^2 = 0.407$; $p = 0.0025$). Although cell-number prechallenge correlated with FEV₁ ($r^2 =$

0.28; $p = 0.02$), there was no significant correlation with mediator levels.

Postallergen challenge. No significant correlations were observed in postallergen challenge between amount of mediators measured and airway reactivity or degree of obstruction. There was no statistical correlation between the size of the immediate wheal observed on allergen skin testing or the amount of allergen instilled and the amount of mediators released after challenge. Likewise, the size and duration of the IAR after the inhalational antigen challenge did not correlate with mediator levels.

DISCUSSION

In the present study, we have demonstrated that a significant difference exists in the amount of mediators measured in the lungs soon after allergen instillation in those subjects with and without an LAR. Previously, we had demonstrated that, in atopic subjects with asthma, histamine, LTC₄, PGD₂, and TX were increased in postallergen challenge compared to increases in control subjects that included normal subjects, atopic subjects without asthma, and nonatopic subjects with asthma. This study further demonstrates that lesser amounts of all four of these mediators are measured in those subjects with an LAR than in those subjects without an LAR.

The significance of increased mediator levels in the airways on the production of an LAR has been controversial. Some investigators have measured small increases in histamine, LTB₄, and LTC₄ in blood or lavage in subjects with LAR,^{21, 22} whereas other investigators have been unable to do so.²³ Several mediators, including histamine, leukotrienes, and TX, have been reported to be increased at the time of the late-phase reaction after nasal-antigen challenge and cutaneous challenge.^{24, 25} Also, pharmacologic studies with a variety of mediator inhibitors have been inconclusive.^{26, 27}

Whether the mediators present at the time of the LAR or the physiologic changes occurring then are at all controlled by events occurring during the IAR is unknown. In this regard, Abraham⁷ proposed that "the late response to inhaled antigen is dependent on mechanisms initiated during the acute allergic reaction." His work was done in sheep with a variety of inhibitors and antagonists. No direct measurement of mediators was performed. However, based on his findings, he believed leukotriene generation during the IAR controlled the LAR. The current study attempted to evaluate that hypothesis in human subjects with asthma and with and without LARs, whereas mediator levels were being directly measured during the time of the IAR.

Although we had demonstrated large increases in mediator levels 5 minutes postendobronchial allergen instillation, there had always been wide ranges of levels measured, with only minimal relation to physiologic parameters during the IAR.⁹ Since there did not appear to be a relationship of mediator levels to the physiologic IAR, we elected to evaluate possible relationships to subsequent LAR production and airway reactivity. It became apparent that, in contrast to speculation based on observations in sheep, lower levels of mediators were measured in subjects with LARs compared to subjects without LARs. Since actual mediator levels were not performed in the studies

summarized by Abraham,⁷ other reasons than differences in mediator levels could explain these results in sheep. Certainly pharmacologic blockers would be effective, regardless of the absolute level of mediators being blocked. The equivalent dose that caused an LAR in dual responding sheep caused only an IAR in single responders, implying that other factors, such as underlying airway reactivity, are also involved.

In a similar fashion, it is well-known that subjects with asthma and with LARs are likely to be more reactive to nonspecific bronchial challenge than those subjects without LAR. This nonspecific increase in airway reactivity to substances such as histamine may imply an alteration in the receptors on the smooth muscle of the airways themselves, or the accessibility of the receptors to the mediators, which allows smaller doses of stimulus to produce the same effects.

Although our results may at first appear unexpected, they are not without precedent. Howarth et al.²⁸ measured histamine in blood after allergen inhalation and was able to demonstrate that the more reactive the airways, the lesser the amount of mediators that was measured in the blood. Similarly, we have demonstrated that, although urinary LTE₄ levels at postallergen challenge relate to the size of the IAR, they are also influenced by the underlying airway reactivity. Again, the more reactive the subject, the less LTE₄ was measured in association with the physiologic changes observed.²⁹ Unfortunately, little was known about the clearance and metabolism of histamine or LTE₄ in these studies; therefore, those more reactive subjects may actually have altered metabolism and clearance rather than decreased production.

The current study extends these results to BALF collected 5 minutes postallergen challenge in which the potential for interference by metabolism may be slightly less. The reasons for the decreased levels of mediators observed in subjects with an LAR may be multitudinal. We were concerned that mediator levels might reflect larger doses of antigen administered, resulting in larger IARs and LARs. To evaluate this concern, we correlated the amount of antigen administered with the size of the IAR and LAR and percent drop in FEV₁. No relationship was observed, and all subjects dropped a similar amount in FEV₁ during the IAR, (–) LAR, 32% to 41% drop in FEV₁; (+) LAR, 23% to 42% drop. There was no difference in skin reactivity to antigen among the groups, with the atopic subjects without asthma actually the most reactive (Table I). Likewise, there was no significant difference between the amount of antigen administered to any of the groups. Correlation analyses between the four mediators and the amount of antigen administered did not demonstrate any significant correlations. In a

similar vein, it is possible that the mediators measured are not important in the LAR.

At a more cellular level, there may be less mast cells present in these individuals to respond to antigen, or the mast cells may be down regulated, perhaps because of chronic antigen exposure. Attempts to quantify mast cells in a previous study were difficult because of the very small numbers of mast cells present in lavage fluid.² Therefore, observing differences in numbers between groups would be impractical. We have no supporting evidence for either down regulation of receptors or decreased releasability of mediators at this stage. Furthermore, no significant differences between the two groups with asthma were demonstrated when evaluation was made for any other cell type. As previously mentioned, it is also possible rapid metabolism and/or clearance of mediators or epithelial permeability changes may decrease the levels of mediators measured. Although the lack of difference in protein values among groups speaks against permeability changes, only kinetic studies of lavage fluid will fully address this issue.

In summary, we have demonstrated clear differences in levels of mediators in BALF found 5 minutes after allergen instillation between subjects with and without LAR. The low levels found in those subjects without LAR would appear to indicate that the physiologic response to allergen challenge is a complex one, requiring mediator production in combination with other, as yet incompletely, defined variable(s), such as airway reactivity, to produce a physiologic LAR. Defining the variables and their relationships in future experiments will be important in furthering our understanding of clinical asthma.

REFERENCES

1. Murray JJ, Tonnel AB, Brash AR. Release of prostaglandin D₂ into human airways during acute allergen challenge. *N Engl J Med* 1986;315:800-4.
2. Wenzel SE, Fowler AA, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge: in vivo release of histamine and tryptase in atopic subjects with and without asthma. *Am Rev Respir Dis* 1988;137:1002-8.
3. Wenzel SE, Westcott JY, Smith HR, Larsen GL. Spectrum of prostanoid release after bronchoalveolar allergen challenge in atopic asthmatics and control groups: an alteration in the ratio of bronchoconstrictive to bronchoprotective mediators. *Am Rev Respir Dis* 1989;139:450-7.
4. Bhat KN, Arroyave CM, Marney SR, Stevenson DD, Tan EM. Plasma histamine changes during provoked bronchospasm in asthmatic patients. *J ALLERGY CLIN IMMUNOL* 1976;58:647-56.
5. Rafferty P, Beasley R, Holgate ST. The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine 5'-monophosphate in atopic asthma. *Am Rev Respir Dis* 1987;136:369-73.
6. Curzen N, Rafferty P, Holgate ST. Effects of a cyclooxygenase inhibitor flubiprofen, and an H₁ receptor antagonist, terfenadine, alone and in combination on allergen-induced immediate bronchoconstriction in man. *Thorax* 1987;42:946-52.
7. Abraham WM. The importance of lipoxygenase products of arachidonic acid in allergen-induced late responses. *Am Rev Respir Dis* 1987;135:S49-S53.
8. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. *J ALLERGY CLIN IMMUNOL* 1987;79:734-40.
9. Wenzel SE, Larsen GL, Johnston K, Voelkel NF, Westcott JY. Elevated levels of leukotriene C₄ in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990;142:112-9.
10. Fick RB, Richerson HB, Zavala DC, Hunninghake GW. Bronchoalveolar lavage in allergic asthmatics. *Am Rev Respir Dis* 1987;135:1204-9.
11. Hargreave FE, Dolovich J, Boulet LP. Inhalation provocation tests. *Semin Respir Med* 1983;4:224-35.
12. Bernstein IL, Boushey HA, Cherniack RM, et al. Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatic patients. *Am Rev Respir Dis* 1985;132:180-2.
13. Markwell MA, Haas SM, Bieber CC, Tolber NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* 1978;87:206-10.
14. Shaff RE, Beaven MA. Increased sensitivity of the enzymatic isotopic assay of histamine in plasma and serum. *Anal Biochem* 1979;94:425-30.
15. Rauls DO, Ting S, Lung M. Analysis of plasma histamine: a modification of the enzymatic isotopic assay. *J ALLERGY CLIN IMMUNOL* 1986;77:673-6.
16. Pradelles P, Grassi J, Maclouf J. Enzyme immunoassays of eicosanoids using acetylcholine esterase as label: an alternative to radioimmunoassay. *Anal Chem* 1985;57:1170-3.
17. Kelly RW, Deam S, Cameron MJ, Seamark RF. Measurement by radioimmunoassay of prostaglandins as their methyl oximes. *Prostaglandins Leukotrienes Med* 1986;24:1-14.
18. Westcott JY, Johnston K, Batt RA, Wenzel SE, Voelkel NF. Measurement of peptidoleukotrienes in biologic fluids. *J Appl Physiol* 1990;68:2640-8.
19. Rosner B. Fundamentals of biostatistics. Boston: Duxbury Press, 1986.
20. O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987;136:740-51.
21. Durham SR, Lee TH, Cromwell O, et al. Immunologic studies in allergen-induced late-phase asthmatic reactions. *J ALLERGY CLIN IMMUNOL* 1984;74:49-60.
22. Diaz PM, Gonzalez C, Galleguillos FR, et al. Leukocytes and mediators in bronchoalveolar lavage during allergen-induced late-phase asthmatic responses. *Am Rev Respir Dis* 1989;139:1383-9.
23. Smith HR, Bethel RA, Cherniack RM, Westcott JY, Voelkel NF, Larsen GL. Bronchoalveolar lavage during the late asthmatic response in man: inflammatory cells, eicosanoid concentrations, change in airway responsiveness. *Am Rev Respir Dis* 1989;139:A464.
24. Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
25. Dorsch W, Ring J, Reimann J, Geiger R. Mediator studies in skin-blister fluid from patients with dual skin reactions af-

- ter intradermal allergen injection. *J ALLERGY CLIN IMMUNOL* 1982;70:236-42.
26. Britton JB, Hanley SP, Tattersfield AE. The effect of an oral leukotriene D₄ antagonist L-649,923 on the response to inhaled antigen in asthma. *J ALLERGY CLIN IMMUNOL* 1987;79:811-6.
 27. Shephard EJ, Malan L, McFarlane CM, Mouton W, Joubert JR. Lung function and plasma levels of thromboxane B₂, 6-ketoprostaglandin F_{1α}, and β-thromboglobulin in antigen-induced asthma before and after indomethacin pretreatment. *Br J Clin Pharmacol* 1985;19:459-70.
 28. Howarth PH, Durham SR, Kay AB, Holgate ST. The relationship between mast cell-mediator release and bronchial reactivity in allergic asthma. *J ALLERGY CLIN IMMUNOL* 1987;80:703-11.
 29. Westcott JY, Smith HR, Wenzel SE, et al. Urinary leukotriene E₄ excretion in patients with asthma: effect of airways reactivity and sodium cromoglycate [submitted].

Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia

Emma Griffin, BSc,* Lena Håkansson, PhD,** Hans Formgren, MD,*
Kirsten Jörgensen, LT,* Christer Peterson, PhD,** and Per Venge, MD**
Åre and Uppsala, Sweden

*Blood eosinophil count, serum concentrations of eosinophil cationic protein (ECP), eosinophil protein X (EPX), myeloperoxidase (MPO), and peak expiratory flow (PEF) rate were studied in 23 patients with severe labile asthma characterized by eosinophilia at the start and end of a treatment period of 5 weeks. The mean blood eosinophil count was $808 \times 10^6/L$ at the start of the treatment period. Serum ECP and EPX were significantly raised compared with that of the references, whereas the mean serum MPO level was normal. The mean PEF was significantly and negatively correlated to both blood eosinophil count and serum ECP and EPX, but the predominant correlation was that between blood eosinophil count and PEF. At the end of the treatment period, PEF had increased and the blood eosinophil count and serum ECP and EPX were reduced when these values were compared with the values at the start of the treatment period. There was a significant and negative correlation of mean PEF to serum ECP but not to the blood eosinophil count. In individual subjects, the decreases in the blood eosinophil counts and serum EPX were significantly correlated to the individual increases of mean PEF. In conclusion, the present investigation indicates that in patients with asthma and pronounced eosinophilia, the lung function of the patients was principally related to the number of circulating eosinophils, whereas, when their eosinophilia was reduced to moderate levels, the patient's lung function was closer related to the activity of the eosinophils. (*J ALLERGY CLIN IMMUNOL* 1991;87:548-57.)*

Eosinophilia is an outstanding feature in patients with asthma.¹ A relationship has previously been demonstrated between the number of eosinophils in the blood and the lung function of the patients.²⁻⁴ Exten-

Abbreviations used

ECP: Eosinophil cationic protein
EDN: Eosinophil-derived neurotoxin
EPO: Eosinophil peroxidase
EPX: Eosinophil protein X
MBP: Major basic protein
MPO: Myeloperoxidase
PEF: Peak expiratory flow

From the *Asthma and Allergy Clinic, Åre Hospital, Åre, Sweden, and **Laboratory for Inflammation Research, Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.

Supported by grants from Th Bergs stiftelse, the Swedish Medical Research Council, the Medical Faculty of the University of Uppsala, and the Swedish National Environment Protection Board.

Received for publication April 24, 1990.

Revised Sept. 24, 1990.

Accepted for publication Oct. 4, 1990.

Reprint requests: Lena Håkansson, PhD, Laboratory for Inflammation Research, Department of Clinical Chemistry, University Hospital, S-751 85 Uppsala, Sweden.

1/1/25933

sive evidence has been presented to suggest that the eosinophil is the major cell responsible for the inflammatory events occurring in asthma. Secretion of granular components is probably of considerable importance for the inflammatory effects of the eosinophils. The major granule constituents are highly basic, cytotoxic proteins: ECP, EPO, EPX or EDN, and MBP.⁵