Inflammatory cells and eicosanoid mediators in subjects with late asthmatic responses and increases in airway responsiveness

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To determine the relationship of inflammatory cells and eicosanoid mediators to the pathogenesis of the late asthmatic response (LAR) and increases in nonspecific airway responsiveness, we studied bronchoalveolar lavage (BAL) cells and fluid in 27 subjects 12 hours after inhaled antigen challenge. Methacholine challenge was performed before antigen challenge and 24 hours later (12 hours after BAL). Eight subjects had no LAR (−LAR, ≤10% fall in FEV_1), nine subjects had an equivocal LAR (±LAR, 11% 25% fall in FEV_1), and 10 subjects had a definite LAR (+LAR, >25% fall in FEV_1). Subjects developing +LAR had increased airway responsiveness at baseline compared with that of subjects developing an ±LAR, but not with subjects having −LAR. If airway responsiveness was markedly increased at baseline, further increases after antigen challenge were often not observed. We found that both percent neutrophils and eosinophils increased in BAL as the severity of the LAR increased, but significant differences between the groups with −LAR and +LAR were only observed when both cell types were considered together. In addition, there was a significant correlation between the combined cell percentages and the severity of the LAR as determined by fall in FEV_1. Likewise, increases in airway responsiveness were associated with significant increases in both neutrophil and eosinophil numbers, but only neutrophils correlated with the change in airway responsiveness after antigen challenge. However, despite the significant physiologic and cellular differences that we found between our groups, no significant differences could be found in BAL eicosanoid-mediator concentrations. These data suggest that both neutrophils and eosinophils are important to the development of the LAR and increased airway hyperresponsiveness. Changes in eicosanoid mediators cannot be detected in BAL fluid after inhaled antigen challenge despite significant physiologic and cellular changes. (J ALLERGY CLIN IMMUNOL 1992;89:1076-84.)

Key words: *Asthma, late asthmatic response, airway hyperresponsiveness, bronchoalveolar lavage, inflammation, mediators*

The LAR to inhaled antigen is associated with airway inflammation and an increase in nonspecific airway responsiveness, two hallmarks of clinical

Abbreviations used

LAR:	Late asthmatic response
BALF:	Bronchoalveolar lavage fluid
−LAR:	No LAR
±LAR:	Equivocal LAR:
+LAR:	Definite LAR
IAR:	Immediate asthmatic response
BAL:	Bronchoalveolar lavage
PC_{20}:	Provocative concentration of methacholine causing a 20% fall in FEV_1
PML:	Polymorphonuclear leukocytes
PGD_2, PGE_2, PGF_2:	Prostaglandins D_2, E_2, F_2
LTC_4:	Leukotriene C_4
TX:	Thromboxane

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asthma.[1] The severity of the LAR depends, in part, on baseline airway caliber, the sensitivity to inhaled antigen,[2] and the dose of antigen. Furthermore, the development of airway inflammation[3-5] and the release of inflammatory mediators appear to be required.[6] The subsequent increase in airway responsiveness depends on the development of inflammation[7] and perhaps on the development of epithelial injury.[8, 9] However, the components of airway inflammation and mediator release that cause an increase in airway responsiveness are not well understood.

The purpose of this study was to determine whether specific cells or eicosanoid mediators of the inflammatory response are associated with the LAR and the increase in nonspecific airway responsiveness that follows. Furthermore, we sought to determine whether an LAR is more likely to occur in subjects having more severe underlying asthma, as reflected by a lower FEV_1 and more nonspecific airway responsiveness at baseline. To accomplish these goals, we performed BAL 12 hours after antigen-inhalation challenge in allergic subjects with asthma with and without LARs. We measured inflammatory cells and eicosanoid-mediator concentrations in BALF and assessed airway reponsiveness to methacholine before and 24 hours after antigen challenge.

METHODS

Study protocol

Twenty-seven antigen-sensitive subjects with asthma came to the pulmonary function laboratory after withholding bronchodilators, as indicated below. After nonspecific airway reponsiveness was measured by methacholine-inhalation challenge, subjects underwent antigen-inhalation challenge. The bronchoconstrictor response to antigen was measured during the subsequent 12 hours. At the end of that time, BAL was performed for measurement of cell counts and eicosanoid-mediator concentrations. Nonspecific airway responsiveness was measured again 24 hours after antigen challenge.

Subjects

Thirteen female and 14 male subjects were selected for this study based on a clinical history of asthma and positive immediate skin test responsiveness to at least one of 13 common inhaled antigens. All subjects had mild asthma at the time of the study, as defined by the ability to withhold β-agonists for at least 6 hours and oral theophylline preparations for at least 12 hours before physiologic testing or antigen challenge. No subject took inhaled or oral corticosteroids. Subjects were excluded from the study if baseline FEV_1 was <60% predicted, if they smoked, if they were receiving immunotherapy, or if they had other significant disease (i.e., congestive heart failure, renal disease, inflammatory bowel disease, etc.). All subjects signed an informed consent form approved by the Institutional Review Board at the National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo., before participating in the study. Skin testing was performed to determine sensitivity to inhaled antigen, with a prick skin test with commercially available antigen extracts at 1:10 or 1:20 dilutions as recommended by the manufacturer. If the skin test response to more than one antigen was positive (wheal, >5 mm at 15 minutes), then the antigen selected for inhalation challenge was determined by clinical history. Antigens included cat, timothy grass, ragweed, *Alternaria*, and Chinese elm. In the group with −LAR, six subjects received cat, one, Chinese elm; and one subject received timothy antigen. In the group with ±LAR, three subjects received cat and seven received timothy. In the group with +LAR, two received cat; four, timothy; two, *Alternaria*; and two received ragweed.

Methacholine challenge

Methacholine challenge was performed according to the methods of Chai et al.[10] Subjects slowly inhaled five inspiratory-capactiy breaths from a DeVilbiss No. 646 nebulizer activated by the patient at the time of inspiration (Sommerset, Pa.). The nebulizer contained, first, normal saline diluent solution and then, doubling concentrations of methacholine starting at a methacholine concentration of 0.01 mg/ml. With a Collins Eagle II spirometer (Braintree, Mass.), FEV_1 was measured at baseline and then 30 seconds and 2 minutes after each inhalation. The lower of these two FEV_1 values was used to calculate PC_{20}. The first methacholine challenge was performed at 8 AM, immediately before antigen challenge. The second methacholine challenge was performed approximately 24 hours after antigen challenge (12 hours after BAL).

Antigen challenge

Antigen challenge was performed with an antigen that previously elicited a positive skin test reaction and that correlated best with a clinical history of either an IAR and/or LAR. When the FEV_1 had returned to within 10% of the baseline after methacholine challenge, antigen inhalation was begun. With a similar DeVilbiss nebulizer, subjects inhaled five times to total lung capacity, starting with an initial concentration of antigen of 1:1,000,000. Spirometry was performed 5 and 15 minutes after the antigen-inhalation challenge. Tenfold increasing concentrations were administered until a fall in FEV_1 of 25% or more was achieved or a concentration of 1:100 was reached. For patient safety, if the FEV_1 dropped between 15% and 20% with a given dose of antigen, then the same dose was repeated instead of administering a tenfold higher concentration. The time of the fall in FEV_1 of 25% or more was recorded and labeled as time zero. Spirometry was performed every 15 minutes for the first 2 hours and then hourly after that time for 10 hours. Subjects in the "definite LAR" group who had a drop in FEV_1 of 40% or more from baseline and who were symptomatic and uncomfortable, were administered albuterol by metered-dose inhaler every 2 hours as needed. No albuterol was administered during the 12 hours after antigen challenge to subjects with no or equivocal LARs.

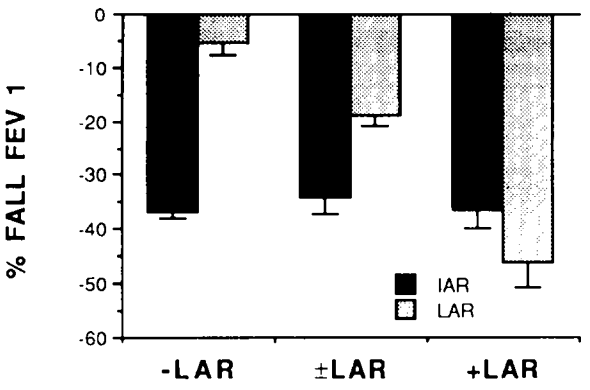

FIG. 1. Differences in percent fall in FEV_1 from baseline for three groups. See text for definitions of IAR and LAR.

BAL

BAL was performed 12 hours after the initial fall in FEV_1. Recommendations of the National Heart, Lung, and Blood Institute for BAL in subjects with asthma were followed.[11] Codeine (30 mg) and atropine (0.6 mg), intramuscularly, midazolam (2 to 5 mg), intravenously, and topical lidocaine were used before and during the procedure as commonly prescribed. Oxygen was administered at 4 L/min by nasal cannulae, and oxygen saturation and heart rate were monitored during the procedure and after the procedure. An Olympus BR4B bronchoscope (Olympus Corp. of America, New Hyde Park, N.Y.) was wedged in the right middle lobe position. BAL was performed with five 60 ml aliquots of warmed sterile saline. After bronchoscopy, the subject was administered nebulized albuterol and was observed overnight.

Lavage fluid was immediately put on ice, and aliquots were combined and centrifuged for 10 minutes at 400 *g* at 4° C to separate cells from supernatant. A part of the supernatants was combined with 2 vol of ice-cold methanol, and both methanol-containing and plain supernatant samples were stored at −70° C for analysis of protein and eicosanoid mediators. Differential cell counts were done from a known volume of lavage with a Diff-Quik (Scientific Products, McGraw Park, Ill.) stain. At least 200 cells were counted to obtain the differential cell count. Cell counts were done with trypan blue exclusion with a hemocytometer. Results were expressed as viable cells per milliliter of BALF.

Three other subjects also underwent bronchoscopy and BAL after methacholine-induced bronchoconstriction to determine inflammatory cell influx and eicosanoid-mediator levels associated with antigen-independent bronchoconstriction. Each subject had a 20% or more fall in FEV_1 from baseline during the methacholine-induced bronchoconstriction.

Mediator analysis

Eicosanoids were analyzed as described previously.[12-14] Briefly, cell-free supernatants of BALF in methanol were partially purified with an octadecylsisyl Supelcoclean cartridge (Supelco, Bellefonte, Pa.), eluted into 5% methanol in ethyl acetate, dried, and resuspended in immunoassay buffer. PGD_2 was measured as the methoxyamine derivative. TXB_2, 6-keto-$PGF_{1\alpha}$, methoxyamine PGD_2, and LTC_4 antisera were generous gifts of Dr. J. Maclouf (INSERM, Paris, France). Enzyme tracer was purchased from AIA Reagents (Aurora, Colo.). Peptidoleukotrienes were segregated with high-performance liquid chromatography and quantified with enzyme immunoassay.[13] The sensitivities of our assays ranged from 5 to 20 pg/ml, depending on the specific mediator. Coefficient of variation ranged between approximately 5% and 15%, with the higher values being at either extreme or the standard curve.[15] All results are expressed as picograms per milliliter of original lavage fluid.

Statistics

Statistical analysis was performed with one-way analysis of variance when more than two data sets were compared, and Student's *t* test was used when two data sets were compared. Associations between variables were assessed by determining linear correlation coefficients. All data are expressed as mean ± SEM.

RESULTS

Physiology

We divided subjects into three groups based on the magnitude of the late asthmatic response: −LAR, ±LAR, and +LAR (Fig. 1). The groups did not differ in the magnitude of the IAR in the first 15 minutes after antigen inhalation: −LAR, 37% ± 1.2% fall in FEV_1; ±LAR, 34% ± 2.9%; +LAR, 36% ± 3.5%. By definition the groups differed in the magnitude of the LAR: −LAR, 5.3% ± 2.5% fall in FEV_1; ±LAR, 19% ± 1.7%; +LAR, 46% ± 4.4% ($p < 0.01$). In addition, subjects with +LAR had persistent decreases in FEV_1 during the LAR that lasted longer than 3 hours, whereas subjects with ±LAR did not. This persistent decrease in FEV_1 was assessed by measurement of the area over the curve described by hourly FEV_1 measurement from 3 to 12 hours. The group with +LAR had a significantly larger area over the curve (95 ± 18 cm^2) compared with that of the group with ±LAR (29 ± 5.1 cm^2) and the group with −LAR (1.1 ± 4.6 cm^2) ($p < 0.01$).

Antigen concentrations required to produce an initial fall in FEV_1 of ≥25% ranged from 1:100 to 1:10,000 for each group, with the exception that one subject in both the group with ±LAR and the group with +LAR received a 1:100,000 dilution. The mean diluations for the groups with −LAR, ±LAR, and +LAR were 1:3025, 1:12,000, and 1:16,210, respectively. These differences were not significant ($p = 0.57$).

Baseline FEV_1 before antigen challenge (percent predicted of normal) did not differ significantly among the three groups (−LAR, 85% ± 5.1%; ±LAR,

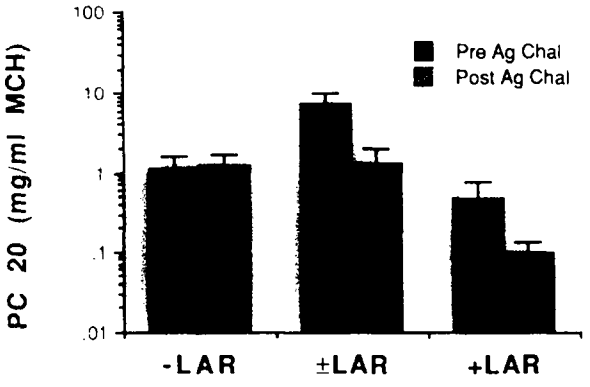

FIG. 2. PC_{20} for three groups 12 hours before antigen challenge *(Pre Ag Chal)* and 12 hours after antigen challenge *(Post Ag Chal)*. See text for significant differences.

86% ± 5.2%; +LAR, 76% ± 3.4%). However, FEV_1 in the group with +LAR tended to be lower.

Baseline PC_{20} before antigen challenge was significantly different for the three groups (Fig. 2). PC_{20} was 1.1 ± 0.46 mg/ml in the group with −LAR, 7.4 ± 2.7 in the group with ±LAR, and 0.5 ± 0.27 in the group with +LAR. The group with ±LAR was significantly less reactive (i.e., a higher PC_{20}) than either the groups with −LAR or +LAR ($p < 0.01$), but the group with +LAR was not more reactive at baseline than the group with −LAR. After antigen-challenge, reactivity did not increase in subjects with −LAR. However, in both the groups with ±LAR and +LAR, reactivity did increase significantly ($p < 0.05$) (Fig. 2). PC_{20} after antigen challenge was 1.2 ± 0.46 mg/ml in the group with −LAR, 1.3 ± 0.72 mg/ml in the group with ±LAR, and 0.1 ± 0.03 mg/ml in the group with +LAR. In addition, despite an overall decrease in PC_{20} in subjects in both the group with ±LAR and the group with +LAR, the PC_{20} of the group with +LAR was significantly less than that of the group with ±LAR after antigen challenge ($p < 0.05$).

In regards to individual airway responsiveness, only one subject in the group with −LAR had a decrease in PC_{20} of twofold or more. In contrast, six of nine subjects in the group with ±LAR and five of 10 subjects in the group with +LAR had decreases in PC_{20} of twofold or more. Of the eight subjects with either a ±LAR or a +LAR who did *not* have a twofold or more decrease in PC_{20}, seven had initial PC_{20}s of 0.5 mg/ml or less, indicating that marked airway hyperresponsiveness was present before antigen challenge. Of the 11 subjects in either the group with ±LAR or group with +LAR who had a PC_{20} of ≤0.5 mg/ml before antigen challenge, seven subjects had less than a twofold decrease in PC_{20}. In contrast, six of seven subjects who had an initial PC_{20} of >0.5 mg/ml had a more than twofold decrease in PC_{20} after antigen challenge.

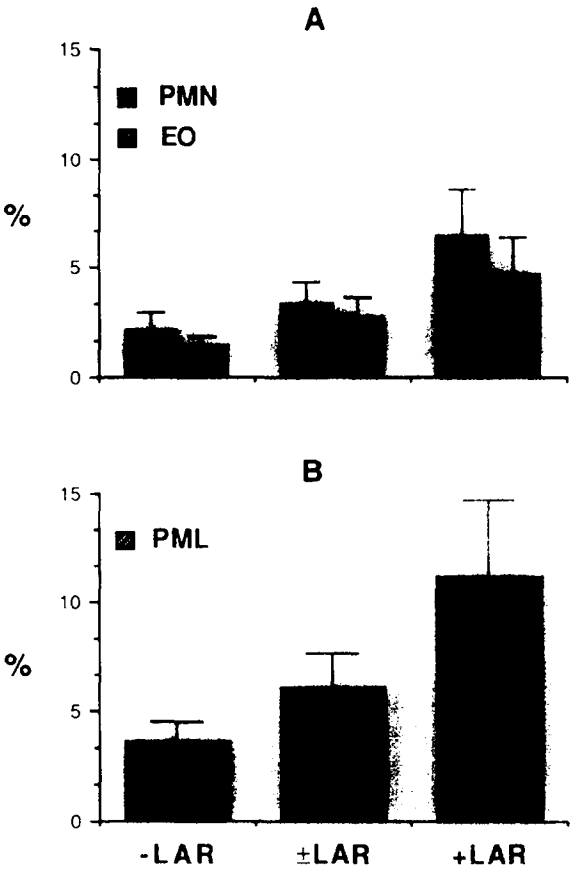

FIG. 3. A, Percent neutrophils *(PMNs)* and eosinophils *(EOs)* in BALF 12 hours after antigen inhalation for three groups. Differences are not significant. **B**, Percent PMLs for three groups. Differences between groups with −LAR and +LAR are significant ($p < 0.05$).

Inflammatory cells

Total cell counts from BALF 12 hours after antigen challenge did not differ significantly among the three groups. Total cells counts × 10^4 were 9.6 ± 1.7 in the group with −LAR, 7.6 ± 1.5 in the group with ±LAR, and 12.5 ± 5.1 in the group with +LAR. Similarly, the percentage of neutrophils or eosinophils did not differ significantly among the three groups at this time point, although percentages of both cell types tended to increase as the severity of the LAR increased (Fig. 3, *A*). For the three groups there were 2.1% ± 0.8%, 3.3% ± 1.0%, and 6.5% ± 2.1% neutrophils, and 1.5% ± 0.3%, 2.8% ± 0.8%, and 4.7% ± 1.6% eosinophils, respectively, in BALF. When the percentages of neutrophils and eosinophils were added to elicit percent PMLs, the group with

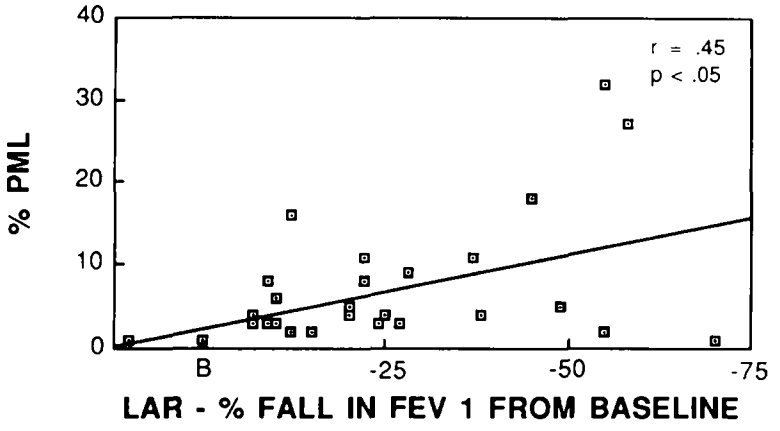

FIG. 4. Correlation between percent PMLs in BALF and magnitude of LAR expressed as percent fall in FEV_1 from baseline; significant correlation.

−LAR and the group with +LAR differed significantly ($p < 0.05$) (Fig. 3, *B*). The intermediate percentage of PMLs of the group with ±LAR did not differ significantly from PMLs of either of the other two groups. There were 3.6% ± 0.8%, 6.1% ± 1.6%, and 11.2% ± 3.5% PMLs, respectively, in the three groups. There was significant overlap in percentages of PMLs among the three groups, with some subjects in the group with +LAR having very low percentages. The magnitude of the fall in FEV_1 during the LAR was found to correlate with the number of PMLs in BALF ($r = 0.45$; $p < 0.05$), suggesting that, as the LAR becomes more severe, the percentage of PMLs increases (Fig. 4).

An increase in airway reactivity after antigen challenge (defined as a twofold or more decrease in PC_{20}) was analyzed in relation to numbers of inflammatory cells in BALF. Percentages of neutrophils, eosinophils, and PMLs did not differ significantly in subjects having an increase in reactivity versus subjects that had no increase. However, when inflammatory cells were expressed as total neutrophils or eosinophils per milliliter, there was a significant difference between the subjects having an increase in reactivity versus subjects that had no increase ($p < 0.05$) (Fig. 5, *A*). The change in PC_{20} was found to correlate with the number of neutrophils per milliliter in BALF ($r = 0.41$; $p < 0.05$) but not with the number of eosinophils or PMLs (Fig. 5, *B*).

There were no significant differences among any of the groups in numbers of macrophages, epithelial cells, lymphocytes, or mast cells in BALF, although special staining techniques were not used to identify specific cellular constituents.

After methacholine-induced bronchoconstriction on three control subjects, inflammatory cells in BALF were not increased (polymorphonuclear neutrophils, 1% to 2%, 0.2×10^4 cells per milliliter; eosinophils, 0% to 2%, 0.14×10^4 cells per milliliter) compared with that of subjects having isolated IARs.

Eicosanoid mediators

Mediator concentrations (picograms per milliliter) in BALF are illustrated in Fig. 6 for TXB_2, PGD_2, PGE_2, and 6-keto-$PGF_{1\alpha}$, a metabolite of prostacyclin. Although the concentrations of the bronchoconstrictor mediators TX and PGD_2 tended to be higher in some subjects with a +LAR than in the other groups, differences were not significant because of the wide scatter of individual data points. Likewise, LTC_4 concentrations performed on BALF from three random patients from each group did not differ among the three groups: −LAR, 132 ± 30; ±LAR, 98 ± 41; +LAR, 194 ± 118 pg/ml. No statistically significant differences were found among groups when mediator concentrations were normalized to BALF protein concentrations; there were no significant differences when mediator concentrations were examined additively or when ratios of "bronchoconstricting" to "bronchodilating" mediators were examined. Total protein concentrations in BALF among the three groups did not differ significantly.

BAL samples from three other subjects with methacholine-induced bronchospasm were also analyzed as controls. Concentration ranges in picograms per milliliter of TX (14 to 184), PGD_2 (34 to 145), PGE_2 (63 to 66), 6-keto-$PGF_{1\alpha}$ (13 to 51), and LTC_4 (22 to 93) overlapped significantly with each of our three groups.

DISCUSSION

This study defines three groups of subjects with asthma in terms of their physiologic response to inhaled antigen. Because there are no absolute definitions as to what constitutes an LAR, we divided sub-

jects into groups based on the severity and duration of the LAR. We believed that to detect an association between LAR, inflammatory cells, mediators, and airway responsiveness, a group of subjects that unequivocally developed +LAR was necessary to compare with a group that developed −LAR. All subject groups had similar immediate bronchoconstrictor responses to inhaled antigen. In contrast, subjects were easily divided into three groups based on the severity of the LAR: subjects with −LAR (fall in FEV_1 <10%), subjects with ±LAR (fall in FEV_1 between 10% to 25%), and subjects with +LAR (fall in FEV_1 of >25%). Furthermore, the magnitude of the LAR as measured by the area above the curve correlated highly with the falls in FEV_1 ($r = 0.89$; $p < 0.001$), confirming the physiologic separation between our three groups.

We first determined whether specific inflammatory cells were associated with the development of an LAR and subsequent increase in airway responsiveness. The association of airway inflammation, as determined by BAL, and the development of the LAR are described in several previous studies. In these studies, both neutrophils and eosinophils have been found[3-5] in increased numbers in BALF after an LAR, but the association of eosinophils has appeared to be stronger, especially at later time points.[4] In this study, both neutrophils and eosinophils were increased in BALF 12 hours after antigen challenge in subjects having +LAR compared with that in subjects having −LAR. There were no significant differences among the three groups when either eosinophils or neutrophils were analyzed individually, although both cell types tended to increase as the severity of the LAR increased. However, when neutrophils and eosinophils were combined as PMLs, there were significantly higher percentages in subjects with +LAR than in subjects with −LAR. In addition, the magnitude of the LAR, as defined by a fall in FEV_1, correlated with the percentage of PMLs. These data imply that both cell types may be important in the inflammatory response associated with the LAR and that there is a direct relationship between severity of inflammation, as indicated by the percent of PMLs and the severity of the LAR. Such a correlation has not been described previously. These findings support the hypothesis that the LAR is chiefly an inflammatory reaction rather than solely a bronchoconstrictive reaction.

When the number of inflammatory cells per milliliter of BALF was compared with the change in airway responsiveness, a significant correlation was found only for neutrophils. However, when twofold increases in responsiveness were analyzed, both neutrophils and eosinophils were significantly increased

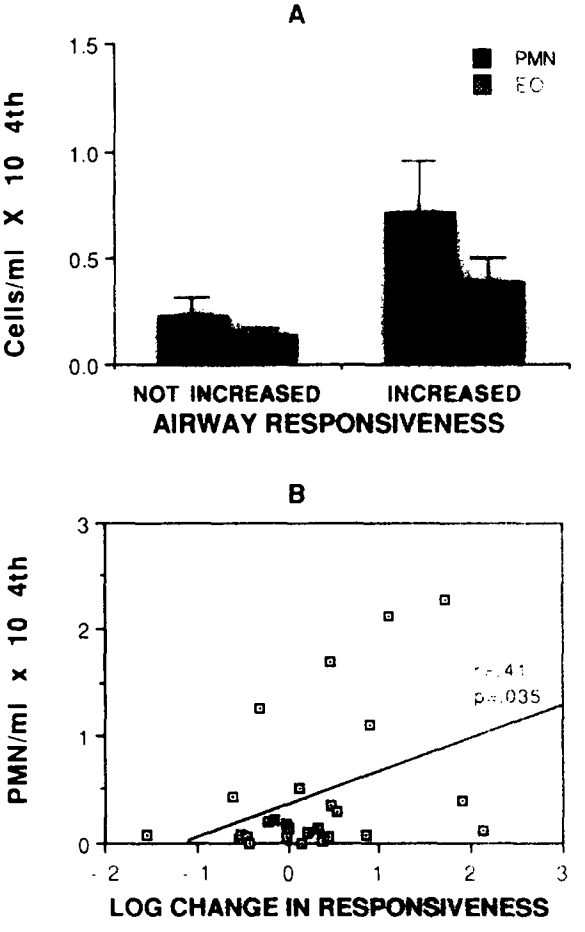

FIG. 5. A, Changes in total numbers of neutrophils *(PMNs)* and eosinophils *(EOs)* for subjects with less than twofold increases in airway reactivity *(not increased)* compared with that of subjects with increases of twofold or more *(increased)*; $p < 0.05$ for both neutrophils and eosinophils. **B**, Correlation between number of neutrophils *(PMNs)* in BALF and change in airway responsiveness to methacholine; significant correlation.

in subjects having twofold or more increases in responsiveness after antigen challenge. Similar correlations and differences were not found with percent eosinophils or neutrophils. These findings support the hypothesis that the increase in airway responsiveness after an LAR is associated with airway inflammation.

Why percent inflammatory cells should be associated with the LAR, whereas absolute numbers of inflammatory cells should be associated with changes in responsiveness, is not clear. Perhaps during the LAR, as cells move into the airway, it is the relative proportion of cells to one another and cell-cell interactions that are important in the production of bronchoconstriction, whereas the total number of cells and their products are important in the changes that lead ultimately to increases in airway reactivity. There was

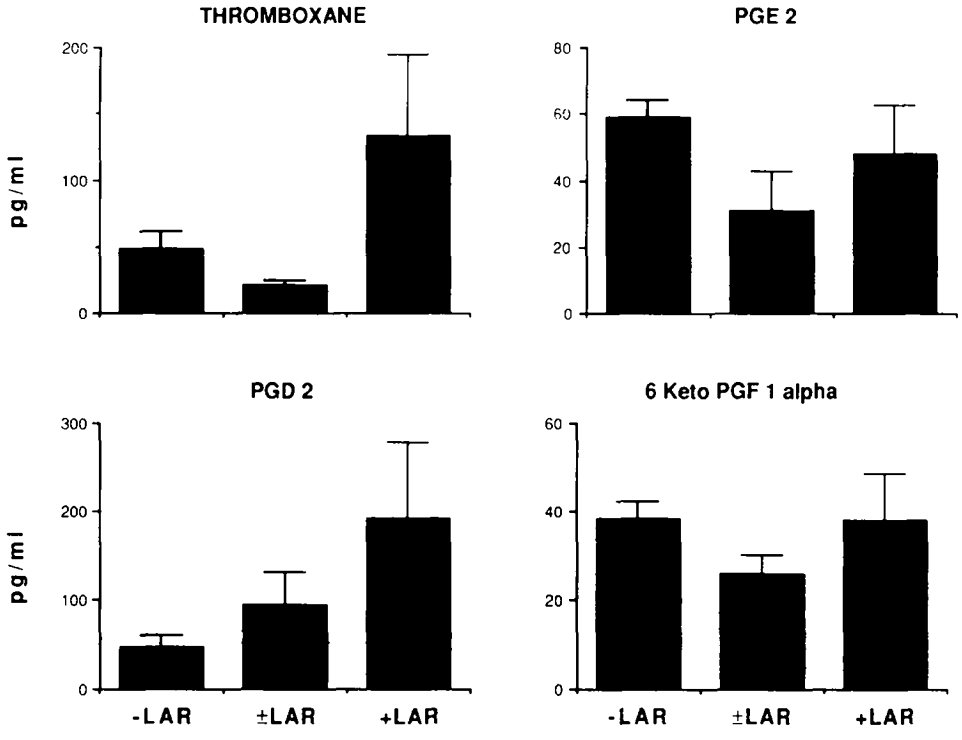

FIG. 6. Changes in TXB_2, PGE_2, PGD_2, and 6-keto-$PGF_{1\alpha}$ concentrations in BALF for three groups; no significant differences for any mediator.

significant heterogeneity among subjects regarding the inflammatory response as reflected in BALF. Some subjects with marked +LARs had <5% PMLs, whereas other subjects had >15%. Similarly, some subjects with −LAR had >5% polymorphonuclear cells in the BALF. Thus, although grouped subjects with LARs and airway hyperresponsiveness have increased inflammatory cells in BALF, this association is not found in all subjects. Perhaps the timing or method of BAL influences the results, or perhaps intraluminal inflammation is not necessary in all patients for the development of LAR.

Second, we determined whether specific eicosanoid mediators of inflammation were associated with the LAR and the subsequent increase in airway responsiveness. When eicosanoid mediator levels in BALF were analyzed, there were no significant differences among the three groups. This finding is especially significant in light of the marked physiologic differences between the groups with −LAR and +LAR. It is unlikely that technical factors in the collection, storage, or analysis of the BALF account for the failure to detect differences among the three groups. There was a tendency for TX, PGD_2, and LTC_4 to be elevated in some patients in the group with +LAR. However, because of the scatter of data, these differences were not statistically significant. Moreover, when the BAL from the three subjects with methacholine-induced bronchospasm were analyzed, there was significant overlap in eicosanoid concentrations between these control subjects and the subjects from all three groups. Thus, despite significant physiologic differences, similar differences were not found in the levels of potential mediators of asthma. These data do not imply that the measured mediators are unimportant in the development of the LAR or the subsequent increase in airway responsiveness. In fact, significant increases in mediator concentrations have been measured soon after local antigen challenge of the airways[7, 14] and in the urine of subjects with more severe LARs.[16] These previous studies and our current data imply that multiple mediators from various cellular constituents, including mast cells (PGD_2), macrophages (LTE_4), and platelets (TX), in addition to neutrophils and eosinophils, may play a role in the development of the LAR. Our data point out that the timing of lavage (12 hours) and/or the route of antigen administration (inhaled) used in this study may not be suitable to detect eicosanoid-mediator signals associated with the LAR or changes in airway responsiveness.

Differences in baseline cellular constituents or base-

line eicosanoid levels are unlikely to have contributed to the airway inflammation and mediator levels that we found 12 hours after antigen challenge. Previous data from our laboratory with the use of slightly different BAL techniques (150 ml total lavage volume versus the 300 ml volume used in this study) have demonstrated no differences in cellular constituents and mediator levels before local antigen challenge in subjects with either −LAR or a +LAR to the particular antigen.[14] These data are directly applicable to our current study despite the protocol differences, because eight of our subjects were among the 12 subjects in the groups with LAR evaluated in the previous study.[14]

Finally, this study protocol allowed us to determine whether characteristics of underlying physiologic or airway responsiveness predict the development of an LAR to inhaled antigen. First, airway caliber, as reflected by percent predicted baseline FEV_1, did not differ significantly among the three groups. However, the lowest percent predicted FEV_1s at baseline were found in the group with +LAR, with four of our 10 subjects having FEV_1 <70% predicted. Although there was a tendency for a lower FEV_1 in the group with +LAR because of the overlap between the groups, this was not significant. Thus, in our study, subjects with more severe underlying airway obstruction as determined by the FEV_1 were not at increased risk to develop an LAR after antigen inhalation.

Likewise, the PC_{20} at baseline did not predict the development of an LAR. Subjects who developed −LAR to inhaled antigen had a PC_{20} of 1.08 ± 4.6 mg/ml, indicating relatively severe underlying airway hyperresponsiveness at baseline, demonstrated more responsiveness than the group with ±LAR. Despite such underlying hyperresponsiveness, the group with −LAR developed no LAR. Because individuals may develop only an immediate response to one antigen but a dual response to another antigen,[17] had we chosen a different antigen for the subjects with −LAR, it is possible that these subjects may have developed a relatively severe LAR. In contrast, subjects that developed ±LAR had significantly higher baseline airway responsiveness than subjects that developed +LAR. This finding implies that for subjects who will go on to an LAR, underlying airway responsiveness is an important determinant of the magnitude of that LAR. These findings are similar to findings of a previous study.[2] In addition, there was a tendency for subjects having the more severe LARs to require less inhaled antigen to generate their decreases in FEV_1, suggesting that their airways are more responsive to antigen. Thus, if a subject with asthma is at risk for an LAR to antigen, the underlying nonspecific airway hyperresponsiveness before antigen exposure may significantly determine the magnitude and severity of the LAR. Although increases in airway reactivity are not addressed in this study as a clinical extrapolation, it would appear reasonable to normalize underlying increases in airway reactivity as much as possible to protect subjects with asthma from the risk of more severe LARs after inadvertent exposure to antigen.

An increase in airway responsiveness after antigen challenge was observed in both subject groups that developed LARs, whereas the group that had −LAR did not develop increased responsiveness. These changes are similar to changes observed by other investigators.[18] Interestingly, however, when individual subjects were analyzed, increases in airway responsiveness after antigen challenge were sometimes not observed in subjects who developed an LAR. Specifically, three of nine subjects in the group with ±LAR and five of 10 patients in the group with +LAR had less than twofold increases in airway responsiveness. Of these eight subjects, seven had baseline PC_{20}s of 0.5 mg/ml or less. This finding suggests that subjects with severe airway hyperresponsiveness at baseline often will not increase responsiveness further after antigen exposure, despite the presence of an LAR. This finding contrasts with the previous study by Cartier et al.[2] but can possibly be explained by differences in baseline responsiveness between subjects of the two studies. Despite no further increase in responsiveness in some subjects, all subjects who had an LAR (either ± or +) were hyperresponsive after antigen challenge (PC_{20}, <1.2 mg/ml). Thus, increases in airway reactivity after an LAR are not necessarily observed if responsiveness is already markedly increased at baseline.

In conclusion, inflammatory responses are observed in association with the LAR and subsequent increases in airway reactivity and consist both of a neutrophilic and eosinophilic influx into the airways. These findings suggest that these inflammatory cells and their products may be important for the development of these two responses. Despite the presence of significant physiologic changes and increases in airway responsiveness, similar changes in specific eicosanoid mediators could not be found 12 hours after antigen inhalation. The use of BAL 12 hours after an inhaled antigen challenge is adequate for identification of cellular components of inflammation associated with the LAR and increases in airway responsiveness, but it is inadequate to detect changes in potentially important inflammatory mediators of asthma. Specific mediators

responsible for the pathophysiologic changes of the LAR and airway hyperresponsiveness need to be elucidated.

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