

Blood markers of early and late airway responses to allergen in asthmatic subjects. Relationship with functional findings

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We evaluated the relationship between blood markers of mast-cell (plasma histamine and serum level of heat-stable neutrophil chemotactic activity [NCA]) and eosinophil (serum eosinophil cationic protein [ECP]) activation during early airway response (EAR) and late airway response (LAR) to allergen inhalation in 24 asthmatic subjects. After EAR, 14 subjects showed significant LAR (FEV_1 fall: $\geq 25\%$), while 10 subjects showed equivocal LAR (FEV_1 fall: 15–20%). A significant increase from baseline value was observed in plasma histamine and in serum NCA during both EAR and LAR, while serum ECP significantly increased only during LAR. The sensitivity of different markers to detect significant FEV_1 fall during EAR and LAR was low, except for NCA. Changes in blood mediators were similar in both groups with significant and equivocal LAR. There was a significant relationship between the increase in NCA during EAR and the severity of LAR. Stepwise regression between changes in different blood markers showed a significant relationship between histamine increase during EAR and ECP increase during LAR. Thus, serum NCA is a more sensitive marker of EAR and LAR than plasma histamine and serum ECP, and its increase during EAR seems predictive of the severity of the subsequent LAR.

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Asthma is defined as a chronic inflammatory disorder in which mast cells and eosinophils play an important role (1). Mast cells are involved in the acute airway response to IgE-mediated stimuli, such as early airway response (EAR) to inhaled allergen in the laboratory (2). Eosinophil recruitment in the airways is found during spontaneous exacerbation of asthma and late airway response (LAR) to allergen (3, 4).

Mast-cell activation during EAR has been demonstrated by the presence of histamine and prostaglandins of mast-cell origin both in bronchoalveolar lavage (BAL) fluid (2, 5) and in serum (6), with a peak at 5–15 min after allergen challenge. Another mediator, a high-molecular-weight factor with neutrophil chemotactic activity (NCA), has been identified in the circulation of asthmatic subjects after early and late airway response to allergen inhalation (7). ECP levels rose in the

serum after late asthmatic response (8), reflecting the degree of activation of the circulating pool of eosinophils.

Therefore, the measurement in the blood of specific markers of various inflammatory cells can be used to detect inflammation in the lung during both EAR and LAR after allergen inhalation. However, the sensitivity of these markers in detecting airway inflammation is not well known. Furthermore, there is general agreement that these mediators increase in the blood some hours after allergen inhalation in subjects with LAR, but not in subjects without LAR (7–9); on the contrary, it is unclear whether changes in blood mediators are related to the severity of LAR. In fact, few data are available on the relationship between the degree of functional airway response and the degree of inflammatory cell activation during allergen challenge.

Our study aimed to assay the sensitivity of some blood markers of mast-cell and eosinophil activation in detecting functional airway response to allergen inhalation in asthmatic subjects. Furthermore, we tried to relate the changes in markers of mast-cell and eosinophil activation to the degree of functional changes during LAR, and to find a relationship between changes in markers of mast-cell activation and changes in markers of eosinophil activation. Then, we selected asthmatic subjects by the presence of allergen-induced LAR of different degree, and we measured mediators of mast-cell or eosinophil origin in the blood at different times during allergen challenge.

Material and methods

Subjects

We examined 24 asthmatic subjects sensitized to *Dermatophagoides pteronyssinus* or grass pollen (17 male and seven female; mean age 24 years [range: 18–54 years]), who had shown, in a previous (screening) specific bronchial provocative test (sBPT), positive EAR to allergen inhalation followed by a late airway FEV₁ fall of variable degree. All subjects were examined in a stable period of the disease and without airway infections during the previous 4 weeks. All subjects had complained of mild asthma symptoms during the previous year; at the time of the study, they showed FEV₁ in normal range, and all but two subjects were hyper-reactive to methacholine (PD₂₀FEV₁ methacholine <1 mg). Although some of them had previously used cromoglycate and inhaled steroids, all subjects were receiving treatment with inhaled salbutamol only as needed in the last month. According to international guidelines (1), asthma was defined as "mild intermittent".

Each patient gave informed consent to participation in the study, which had the approval of the local Ethical Advisory Committee.

Study design

During the study, the subject came to the laboratory for 3 consecutive days. On the first day, in the morning, the subject underwent a methacholine challenge test (MTC1).

On the second day, in the morning, an intravenous cannula was inserted in the antecubital vein. After a 30-min rest, sBPT with allergen was performed: the allergen inhalation was continued until the onset of EAR (FEV₁ fall $\geq 20\%$); then, FEV₁ was monitored in order to determine the appearance of the LAR. Venous blood samples to measure plasma histamine, serum NCA, and ECP were

obtained before the beginning of allergen inhalation and 5, 10, 15, and 30 min after the last dose of allergen (during EAR); then, they were repeated 2 h after allergen inhalation. At the onset of LAR (when FEV₁ fell by 15–20% from the postdiluent value, usually 5–6 h after allergen inhalation), a venous blood sample was collected. Afterward, a further blood sample was taken when a "plateau" in FEV₁ fall was obtained (FEV₁ change in two following measurements at hourly intervals was less than 5%), usually 7–8 h after allergen inhalation. Immediately after the last blood sample, the methacholine challenge test was repeated (MTC2) in subjects with FEV₁ greater than 50% of the predicted value (17/24 subjects).

On the third day (24 h after allergen inhalation), FEV₁ was measured and another venous blood sample was collected. The methacholine challenge test was repeated (MTC3) in all but four subjects who showed persistence of severe LAR (FEV₁ lower than 50% of the predicted value).

In order to control for the spontaneous daily variations of blood markers, the first seven subjects repeated sBPT with inhalation of placebo (diluent solution) on a different day, at least 4 weeks after allergen challenge and in the absence of any changes in clinical findings of asthma. Pulmonary function and blood-marker measurement (histamine, NCA, ECP) was performed at the same time points of the day of allergen challenge.

Methods

Specific bronchial provocative test with allergen. sBPT was performed with allergen standardized in biologic units (BU), as previously described (10). Allergen extract solution was delivered by a De Vilbiss 646 jet nebulizer using the same procedure as for the methacholine inhalation test. Lyophilized allergen extract (NeoAbello, Milan, Italy) was diluted in saline in order to obtain two working solutions with different concentrations (1 and 10 BU/ml). The nebulizer was filled with 3 ml of diluent (phenol 0.4% in saline) or allergen solution. After baseline spirometry, the patient inhaled three puffs of diluent, followed at 10-min intervals by inhalations of cumulative doses of allergen that were, at each step, 0.02, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 BU. FEV₁ was measured 10 min after the end of each series of allergen inhalations by a Biomedin water-sealed spirometer, connected to an Olivetti 240 computer. The inhalations were continued until FEV₁ fell more than 20% below the postdiluent value or until the top concentration had been administered, and the total dose (TD) of allergen delivered into the airways was calculated. FEV₁ was then measured at 20, 30, and 60 min and

then hourly for 8 h. The dose of allergen causing 20% fall in FEV₁ from postdiluent value (PD₂₀FEV₁ allergen) and maximum percent FEV₁ fall at 3–8 h after challenge were used to evaluate the degree of EAR and LAR, respectively. The late bronchoconstrictor response was described also as the area under the FEV₁ time curve at 3–8 h after challenge, derived by trapezoid integration.

Methacholine challenge test. Methacholine was delivered by a De Vilbiss 646 jet nebulizer using a procedure like that of the allergen inhalation test (11). After phosphate-buffered saline inhalation, methacholine was inhaled every 2 min; the cumulative delivered doses at each step were 0.04, 0.12, 0.24, 0.4, 0.8, 1.6, and 3.2 mg of methacholine. The response was measured 2 min after the end of each series of methacholine inhalations, which were continued until FEV₁ fell more than 20% below the postdiluent value or until the top concentration had been administered. Methacholine PD₂₀FEV₁ was calculated by log interpolation from the dose-response curve.

Measurement of mediators in the blood: histamine. A volume of 5 ml of venous blood was put into a chilled polypropylene tube containing 50 µl of 0.5 M EDTA (pH 7.45). This was kept in ice, and the plasma was separated, within 20 min after blood collection, by centrifugation at 900 g at 4°C for 10 min. After separation, two aliquots of the uppermost 300 µl of plasma were aspirated to avoid basophil contamination from the underlying leukocyte coat. Plasma samples were frozen at –80°C until assay.

Histamine was measured by a modified radioenzymatic assay (Immunotech, Marseille, France). The assay is based on the competition between the histamine of the sample, modified by acylation, and ¹²⁵I-labeled histamine, for the binding to high-affinity monoclonal antibody coated on the polypropylene tube. The assay is very specific and sensitive (the lowest measurable concentration: 0.02 ng/ml), with high intra- and interassay reproducibility: 7% and 11%, respectively. Normal values of plasma histamine have been reported to be under 1 ng/ml (12).

Neutrophil chemotactic activity (NCA). A volume of 10 ml of venous blood was put into a tube at room temperature for 2 h; the serum was then separated by centrifugation at 2000 g for 15 min, aspirated, and stored at –80°C.

Blood from normal donors was treated by means of sedimentation with 2% dextran solution (1:1) and centrifugation; then, neutrophils were counted in a Bürker chamber and diluted with Gey's solution to

obtain a final solution of 1.5×10^6 neutrophils/ml, with a purity of $87.5 \pm 5\%$.

Before testing, sera of asthmatic subjects and a pool serum of healthy subjects were heated for 30 min at 56°C to inactivate the complement. Sera were diluted 1:40 with Gey's solution, and measured in triplicate. As control, in each experiment, random migration (spontaneous migration of neutrophils toward Gey's solution, in the absence of bovine serum albumin [BSA], in cellular suspension), chemokinesis (migration toward Gey's solution in the presence of BSA in the cellular suspension), and chemotaxis (migration toward fMLP 10^{-8} M) were measured.

Migration of healthy donors' neutrophils was measured by means of a 48-well microchamber (Neuro Probe; Cabin John, MA, USA). Neuro Probe nitrocellulose filters of 130-µm thickness with 5-µm pore size were placed between the bottom plate and the gasket. In the lower compartment, patient sera, pool of normal sera, Gey's solution (random migration or chemokinesis), or fMLP (chemotaxis) was placed. The neutrophil suspension (50 µl) was added to the top chamber, and the apparatus was incubated for 45 min at 37°C in 5% CO₂. At the end of incubation, the chamber was disassembled, and the removed filters were fixed and stained with hematoxylin. Migration was measured by the leading front technique on at least 10 low-power fields. All blood samples of each subject were evaluated in the same assay, and the intra-assay reproducibility of the test was 4%.

Eosinophilic cationic protein (ECP). A volume of 6 ml of whole blood was sedimented for 1 h at room temperature and then separated by centrifugation at 1500 g for 10 min; the serum samples were stored at –80°C until the assay.

ECP was measured by specific radioimmuno-logic method (ECP RIA, Pharmacia, Uppsala, Sweden); this method has been proven highly specific and sensitive (2 µg/l) with high intra- and interassay reproducibility. The normal value is under 15 µg/l (13).

Statistical analysis

PD₂₀FEV₁ methacholine, PD₂₀FEV₁ allergen, and the TD of allergen administered during sBPT were expressed as geometric means; logarithmic transformation was done for these indices. Changes in methacholine responsiveness after allergen inhalation were expressed as the ratio of baseline PD₂₀FEV₁ methacholine (MTC1) to PD₂₀FEV₁ methacholine during LAR (MTC2) or 24 h after LAR (MTC3). Values of blood mediators were expressed as mean ± standard deviation. Changes in

plasma histamine and serum ECP after allergen challenge were expressed as difference between baseline value and peak value during EAR (blood samples from 5–30 min after allergen challenge) and LAR (blood samples from the beginning of LAR to 24 h after allergen challenge). NCA changes were expressed as percent increase from baseline value.

For determination of the spontaneous daily variability of each mediator in asthmatic subjects, a cutoff value (mean+2 SD) was derived by all blood measurements obtained at different time points in seven asthmatic subjects who underwent a challenge test with placebo, and this value was used to detect a significant change after allergen inhalation.

Statistical analysis was performed with parametric tests (*t*-paired test and ANOVA for two or more groups of repeated measures, respectively) for changes in FEV₁ and methacholine responsiveness, and nonparametric tests (Wilcoxon and Friedman tests for two or more groups of repeated measures, respectively, or the Mann-Whitney U test for unpaired measurements) for absolute values and percent changes of blood mediators. The comparison of changes in blood mediators after allergen inhalation between subjects with different FEV₁ fall during LAR was done by multivariate analysis by ANOVA. Linear regression between changes in blood markers during airway response and changes in FEV₁ during LAR were computed by Spearman's rank correlation. Stepwise regression was used to evaluate the relationship between changes in the different mediators after allergen inhalation. A level of probability lower than 5% was considered significant (14).

Results

Allergen inhalation induced EAR in all subjects, with a mean FEV₁ fall of 42.2±9.4%. Fourteen of 24 subjects showed also significant LAR (maximum FEV₁ fall >25%), while equivocal LAR (maximum FEV₁ fall: 15–20%) was observed in 10 subjects (Table 1). The severity of EAR and the provocative dose of inhaled allergen were similar in the two groups. The area under curve at 3–8 h after allergen challenge was significantly higher in subjects with significant LAR than in subjects with equivocal LAR. Methacholine responsiveness significantly increased during LAR and 24 h after allergen challenge compared to baseline values in subjects with significant LAR, but MTC2/MTC1 and MTC3/MTC1 ratios were not significantly different between the two groups (Table 1).

All subjects showed baseline mean values of plasma histamine in the normal range (0.22±0.09 ng/ml). During sBPT, plasma histamine significantly

Table 1. Mean clinical and functional characteristics of 24 asthmatic subjects with significant or equivocal late airway response to allergen inhalation

	Significant LAR (Δ FEV ₁ : >25%)	Equivocal LAR (Δ FEV ₁ : 15–20%)
<i>n</i>	14	10
Sex	6 F, 8 M	1 F, 9 M
Age (years) (M±SD)	22±5	27±10
Asthma duration (years) (M±SD)	13±9	8±6
Baseline FEV ₁ (% pred) (M±SD)	91±9	93±10
Allergen PD ₂₀ FEV ₁ (GM, BU)	0.06	0.08
EAR (Δ FEV ₁ %) (M±SD)	41.5±10.0	40.1±9.7
LAR (Δ FEV ₁ %) (M±SD)	43.1±8.1	17.1±2.8*
AUC-LAR (M±SD)	108.7±42.2	36.9±13*
PD ₂₀ FEV ₁ (MTC1) (GM, mg)	0.13	0.19
PD ₂₀ FEV ₁ (MTC2) (GM, mg)	0.03**	0.07
PD ₂₀ FEV ₁ (MTC3) (GM, mg)	0.06**	0.1
MTC2/MTC1 ratio	9.6±9.6	6.0±9.0
MTC3/MTC1 ratio	6.4±8.4	2.6±1.9

*Significant difference between two groups, $P<0.001$.

**Significantly different from MTC1.

EAR=early airway response; LAR=late airway response; AUC=area under curve; M±SD=mean±standard deviation; GM=geometric mean; MTC1=baseline methacholine test; MTC2=methacholine test during LAR; MTC3=methacholine test at 24 h after sBPT.

increased at each time point during EAR and LAR in both groups with significant and equivocal LAR (Fig. 1). There was no significant difference between the peak values during EAR and LAR in both groups. Changes from baseline in plasma histamine were not significantly different between subjects with significant and equivocal LAR both during EAR and during LAR (multivariate analysis by ANOVA, $F=0.035$, NS). There was no relationship between changes in plasma histamine and the severity of LAR, as expressed by maximum FEV₁ percent fall or area under curve. When a cutoff value of 0.68 ng/ml was used to detect a significant change in plasma histamine after allergen inhalation, 7/24 subjects showed such increase during EAR and five subjects during LAR (four with significant and one with equivocal LAR).

Serum NCA increased significantly from the baseline value during EAR and at different time points during LAR in both groups of subjects, without any difference between the two groups with different severity of LAR (multivariate analysis by ANOVA, $F=1.35$, NS) (Fig. 2). A significant correlation was observed between NCA percent increase during EAR and the maximum FEV₁ percent fall during LAR ($r=0.5$, $P=0.02$) (Fig. 3) or area under curve during LAR ($r=0.48$, $P=0.03$), while no correlation was observed between NCA changes during LAR and severity of LAR. A 10% change in serum NCA after allergen challenge (limit value of spontaneous variability after placebo inhalation) was observed in 20

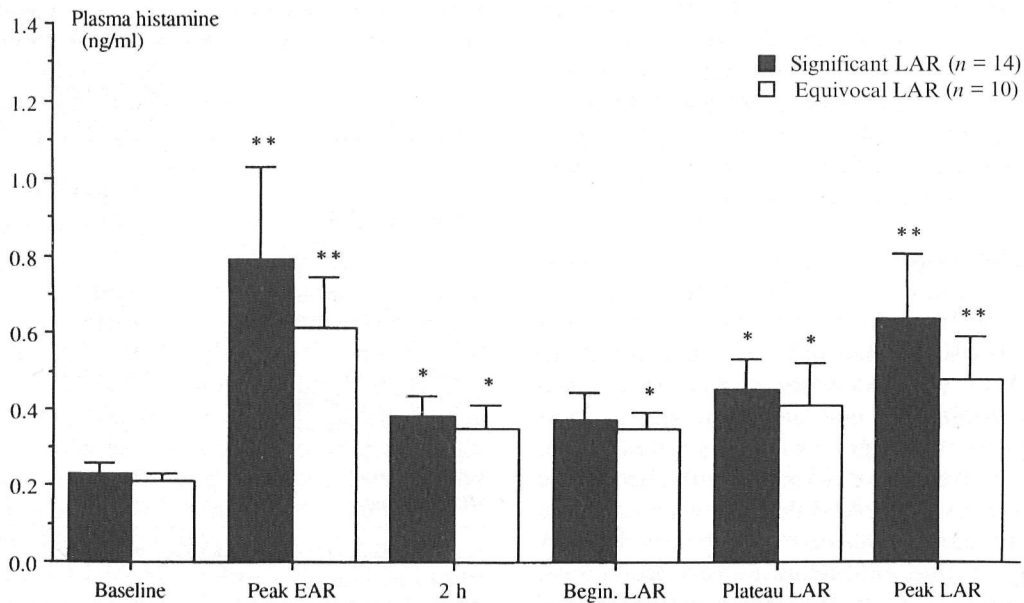


Fig. 1. Mean values (plus standard error) of plasma histamine before and at different time points after allergen inhalation in asthmatic subjects with significant (solid bars) or equivocal (open bars) LAR. Significant difference from baseline value (** $P < 0.01$, * $P < 0.05$).

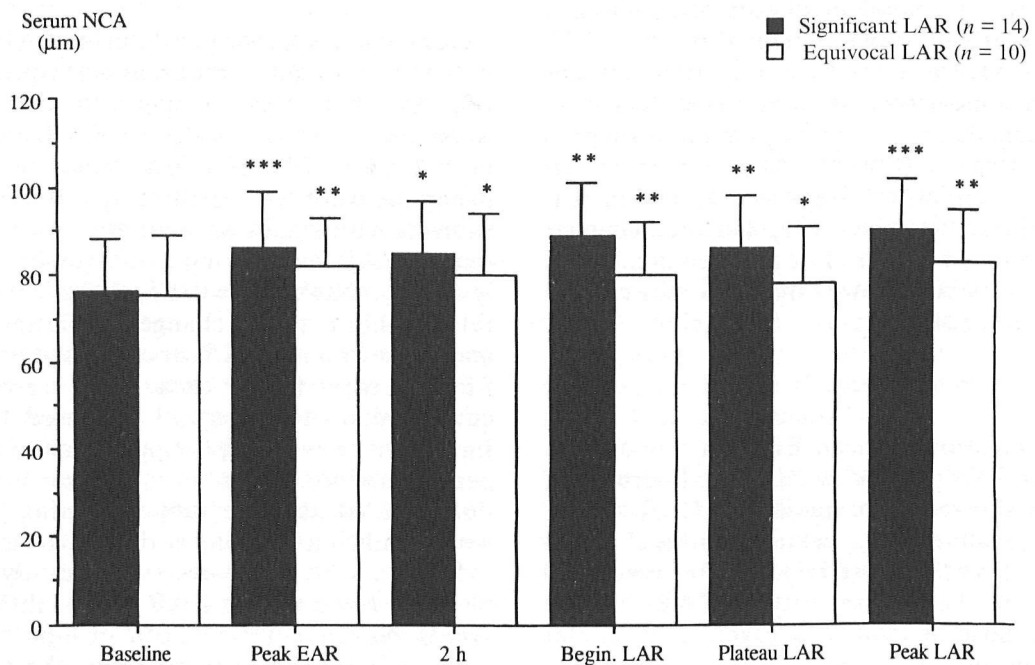


Fig. 2. Mean values (plus standard error) of serum NCA before and at different time points after allergen inhalation in asthmatic subjects with significant (solid bars) or equivocal (open bars) LAR. Significant difference from baseline value (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

subjects during EAR (14 with significant and six with equivocal LAR) and in 22 subjects during LAR (14 with significant and eight with equivocal LAR).

Baseline serum ECP was in the normal range in 18/24 subjects. A significant increase in serum ECP levels after allergen challenge could be observed in

both groups only when the peak value during LAR was considered (Fig. 4). The changes from baseline in ECP levels were not different between the two groups (multivariate analysis by ANOVA, $F = 2.64$, $P = 0.11$), and no correlation was observed between changes in ECP levels and the severity of LAR. With a cutoff value of 14 μg/l, only six subjects

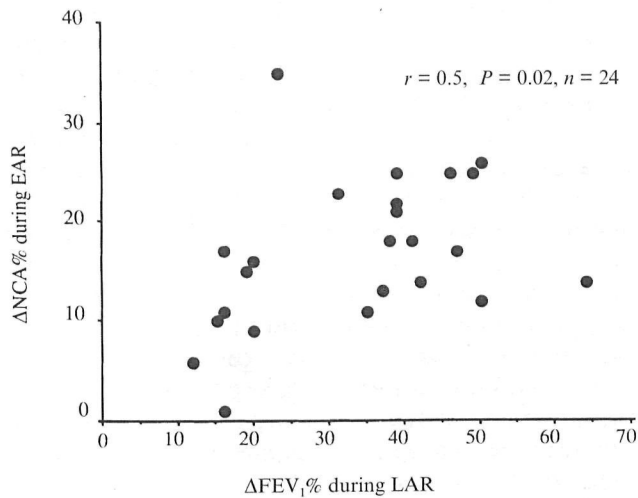


Fig. 3. Relationship between changes in serum NCA (Δ NCA%: percent increase from baseline value) during EAR and maximum fall in FEV₁ (Δ FEV₁%) during LAR.

showed an increase in ECP level during EAR, and 12 during LAR (seven with significant and five with equivocal LAR).

The relationship between the changes in different blood markers during EAR or LAR were also evaluated. The stepwise regression between changes in blood mediators during LAR and during EAR showed a significant correlation between changes in ECP during LAR and changes in histamine during EAR ($F = 11.6$, $P = 0.003$) (Fig. 5);

however, the relationship was not significant when one point with the highest value of change in blood mediator was removed.

Discussion

Our study confirms that some markers of activation of inflammatory cells are involved in the airway response to allergen increase in the blood during early and late asthmatic reactions to allergen. Previous studies reported the measurement of single mediators, such as histamine (6), NCA (7), and ECP (8), in the blood at different time points after allergen inhalation in asthmatic subjects, but our study considered all such mediators simultaneously, in order to evaluate the relative sensitivity of these markers in detecting the underlying airway inflammation. Although changes in these mediators are due to different pathophysiologic mechanisms, our study suggests that NCA is more sensitive than plasma histamine and serum ECP in detecting the activation of inflammatory cells related to airway response in the first 24 h after allergen inhalation. Furthermore, our study shows that there is a poor relationship between these markers of airway inflammation and the severity of the functional response to allergen inhalation. Only the increase in serum NCA during EAR is predictive of severe LAR.

Airway response to allergen in the laboratory, particularly LAR, has been considered to mimic spontaneous asthma (15), and it is associated with

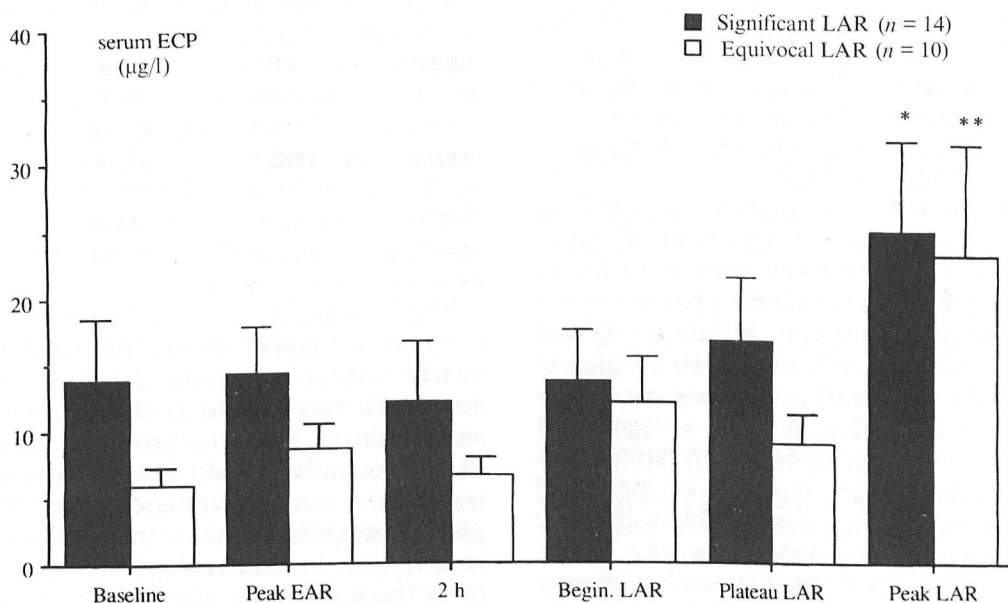


Fig. 4. Mean values (plus standard error) of serum ECP before and at different time points after allergen inhalation in asthmatic subjects with significant (solid bars) or equivocal (open bars) LAR. Significant difference from baseline value (** $P < 0.01$, * $P < 0.05$).

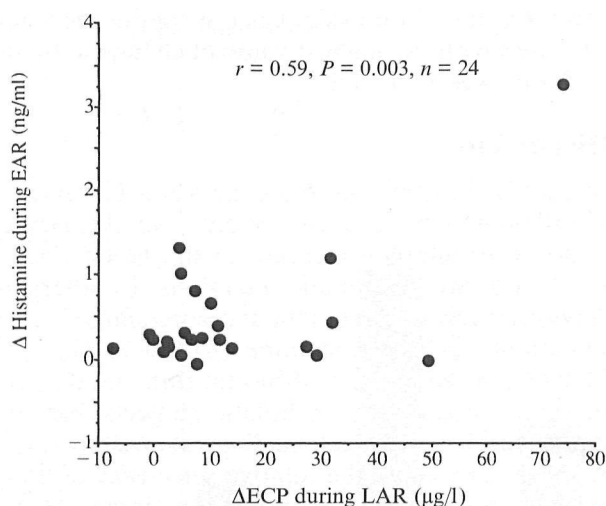


Fig. 5. Relationship between changes in plasma histamine during EAR (expressed as difference between peak value during EAR and baseline value) and changes in serum ECP during LAR (expressed as difference between peak value during LAR and baseline value).

activation of resident lung cells (mast cells and alveolar macrophages), as well as of other inflammatory cells recruited from the blood (eosinophils, lymphocytes, monocytes, and neutrophils) (16). The role of the different cell populations in determining the severity of bronchoconstriction is difficult to define, considering the contribution of other factors, such as airway wall edema due to increased vascular permeability (17), or the sensitivity of bronchial smooth muscle to the constricting stimuli (18). Our study was planned to assess whether a higher degree of changes in blood markers could be observed in subjects with severe LAR to the allergen than in subjects with equivocal LAR. We did not observe any difference in the degree of changes in blood markers between these two asthmatic groups, suggesting that factors other than the severity of inflammatory cell activation are responsible for the severity of LAR.

Plasma histamine has been shown to increase during allergen challenge, but the obtained results were influenced by the sensitivity of the analytic procedure, possible contamination by basophils, and spontaneous variation of plasma histamine. Recent studies have confirmed that low levels of plasma histamine can be measured in baseline conditions in mildly asthmatic subjects, that a significant increase can be observed immediately after allergen inhalation, and that the blood levels measured after allergen inhalation are comparable with those observed after histamine inhalation (6). These observations suggest that the increase in plasma histamine is probably due to some "leakage" in the blood of the histamine released by mast cells in the

airways after allergen challenge. More controversial are the data on the changes in plasma histamine during LAR (6, 9). In a subgroup of our subjects, we obtained a measure of the spontaneous variability of plasma histamine during the day, and we used the confidence limit of this variation to define the presence of a significant change in plasma histamine during LAR; therefore, we can exclude the hypothesis that the increase in plasma histamine during LAR was due to the spontaneous daily variation of this marker. The significance of this increase has not been explained. Our data show the lack of a significant relationship between the degree of airway responsiveness to allergen and the increase in plasma histamine after allergen inhalation.

Serum NCA showed the highest sensitivity in detecting the presence of significant airway response to allergen inhalation; in fact, almost all subjects showed significant increase in serum NCA during both EAR and LAR, independently of the severity of the functional response. Previous authors reported that serum NCA increased after allergen challenge (7, 19), but most studies did not report any relationship between the degree of NCA increase and the severity of FEV₁ fall after different challenges including allergen challenge (10, 20, 21). Only Sastre et al. reported in a small group of subjects a relationship between FEV₁ fall and increase in NCA during EAR to toluene-diisocyanate inhalation (22). In our study, we observed that a higher increase in NCA during EAR was predictive of more severe FEV₁ fall during the subsequent LAR. This fact, which has not been previously reported, suggests that early events occurring immediately after allergen inhalation can influence the development of LAR. Although the chemical characterization and the cellular origin of NCA have not been defined (from mast cells or from blood monocytes activated by mast-cell-derived mediators), we can assume that NCA reflects mast-cell activation after allergen challenge; therefore, our data suggest that the severity of LAR is related to a different pattern of mast-cell activation.

The increase in blood NCA after allergen challenge may suggest a role for neutrophils in the airway response to allergen inhalation. While eosinophil recruitment is always observed in the airways during LAR, neutrophils have been shown to increase in BAL fluid during LAR by some (23), but not by other, investigators (4). In the stable phase, asthmatic subjects show neutrophil counts in BAL or in bronchial biopsy that are not different from those found in normal subjects (24). Therefore, the increase in NCA after allergen challenge should be considered a marker of mast-cell activa-

tion, but it cannot play a significant role in the recruitment of inflammatory cells in the airways.

ECP levels in the serum and in bronchoalveolar lavage (BAL) fluid have been used as a marker of airway inflammation in chronic asthma, both in children and in adults (25–27). Serum ECP is assumed to derive from activated eosinophils in the blood, and not directly from the airways. It has been demonstrated that airway inflammation is associated with release of mediators able to induce eosinophil proliferation and their activation in the blood (28). During allergen challenge in mildly asthmatic subjects, eosinophils decrease in the blood after 6–8 h, and increase after 24–48 h (29). Serum ECP levels paralleled these changes (8, 30). In our data, the sensitivity of this marker to detect late airway bronchoconstriction was fairly low, without any apparent relationship to the severity of the response. This observation, which has not been previously reported, confirms that serum ECP is more useful to monitor chronic airway inflammation than acute airway response to allergen. However, the extension of the measurement of ECP to 48–72 h after allergen challenge could improve the sensitivity of this marker.

Finally, we observed a slight relationship between the increase in serum ECP during LAR and the increase in plasma histamine during EAR. The events leading to LAR after EAR are not known. Some data indicate an important role for mast cells in eosinophil infiltration into the lungs. Mast cells contain various mediators and cytokines, chemotactic for eosinophils, which, upon release after allergen challenge, activate and recruit eosinophils. These include histamine, lipid mediators such as leukotriene B₄ and platelet-activating factor (PAF), and various proinflammatory cytokines such as interleukin (IL)-3, IL-5, IL-4, tumor necrosis factor, and GM-CSF (5). A recent study identifies an important role for mast cells in the eosinophil infiltration into the lungs of allergic mice (31). Preliminary data suggest that inhibition of early mast-cell activation after allergen inhalation by sodium nedocromil can reduce the influx of eosinophils in the airways 48 h later (32). There is also evidence that in asthmatic subjects selected T-cell populations are activated, with subsequent recruitment of eosinophils by specific cytokine release (33). Furthermore, some authors have shown that increased levels of circulating eosinophils and local accumulation of eosinophils at the sites of acute or chronic inflammation occur through release of mast-cell factors. Histamine and PAF derived from mast cells induce, respectively, P-selectin-dependent leukocyte rolling and CD18-dependent leukocyte adhesion to endothelium, confirming a role for mast cells in eosinophil recruitment (34).

We conclude that markers of inflammatory cell activation during EAR seem predictive of the severity of the subsequent LAR and the following activation of blood eosinophils. However, the poor relationship between the degree of the LAR after allergen challenge and the changes in markers of mast-cell and eosinophil activation suggest that other factors, such as airway-wall edema and smooth-muscle contractility, are important determinants of the severity of late response. The mechanisms involved in linking allergen-induced EAR to LAR in the laboratory need further investigation.

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