

The effect of inhaled allergen on circulating basophils in atopic asthma

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*There is increasing evidence for the role of basophils in the allergen-induced late asthmatic response (LAR). To study the effect of inhaled allergen on basophil function in subjects with asthma, ex vivo basophil spontaneous histamine release (SHR) in peripheral blood and plasma histamine was measured before and 2, 5, 10, and 15 minutes, and 2, 4, 6, and 8 hours after allergen bronchial challenge (allergen study day) in six subjects with atopic asthma. Allergen inhalation induced an early response and LAR consisting of a mean ($\pm$ SD) 32.5% ($\pm$ 7.9%) and 28.8% ($\pm$ 7.7%) fall in FEV₁, respectively. As a control for the effects of bronchoconstriction, on another occasion, methacholine challenge was performed to produce a mean 33.4% ($\pm$ 3.4%) fall in FEV₁ during the early response and no LAR, and blood was obtained to measure basophil histamine release (HR) and plasma histamine. There was a small, but significant ($p < 0.05$), rise in median SHR from 4.6% to 6.1% of total basophil histamine after allergen but not after methacholine inhalation. HR remained high after allergen inhalation during the 8 hours of study, whereas it demonstrated a steady, significant, decrease between 4 to 8 hours after methacholine inhalation. No significant changes in plasma histamine were recorded on either allergen or methacholine study days. On a third occasion, SHR was measured after challenge with physiologic saline to control for any effects of methacholine on SHR, and a decrease in HR was recorded during the day similar to HR observed after methacholine challenge. These studies suggest an enhancing effect of inhaled allergen on SHR. The increase in SHR *ex vivo* is not necessarily associated with increased plasma histamine levels *in vivo* and may be more a reflection of basophil activation, possibly involving histamine-releasing factors and cytokines. (J ALLERGY CLIN IMMUNOL 1992;90:175-83.)*

Key words: Asthma, basophils, histamine, bronchial provocation tests, allergens, methacholine chloride

The basophil leukocyte is considered to be relevant to allergic disease since it is the circulating equivalent of the tissue mast cell and possesses high-affinity receptors for IgE (Fc ϵ R₁) on its cell surface.¹ There is increasing evidence for the involvement of this cell in allergic airway disease. Local allergen challenge in either the nose² or lower airways³ is associated with a late response characterized by increased recovery of metachromatic cells from the mucosal surface. These

Abbreviations used

SHR:	Spontaneous histamine release
LAR:	Late asthmatic reaction
EAR:	Early asthmatic reaction
PC ₂₀ methacholine:	Provocative concentration of methacholine causing a 20% in FEV ₁
PC ₂₀ allergen:	Provocative concentration of allergen causing a fall in FEV ₁ >20% and <40% from baseline
HEPES:	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HBSG:	HEPES-buffered saline glucose
AUC:	Area under the curve
IL:	Interleukin
HRF:	Histamine-releasing factor

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cells have features of basophils and are in an activated state, with evidence of increased SHR in the lungs.³ Their appearance in the nose is associated with ele-

TABLE I. Characteristics of subjects

Subject No.	Age (yr)	Allergen	Total IgE (IU)	Specific IgE (cpm)	PC ₂₀ (all) (mg/ml)	PC ₂₀ (M) (mg/ml)	FEV ₁ (All*) (% pred)	FEV ₁ (M†) (% pred)
1	23	Dp	454	6355	0.02	0.38	93.9	115.2
2	22	Dp	35	1625	0.24	6.28	110.9	108.60
3	30	Dp	5952	5974	0.24	2.21	83.3	87.9
4	27	GP	2624	4792	1.25	0.41	118.8	118.8
5	19	Dp	81	4384	0.12	0.80	114.3	107.4
6	21	Dp	5376	6555	0.012	0.13	73.9	65.2

All, Allergen; M, methacholine; % pred, percent predicted; Dp, *Dermatophagoides pteronyssinus*, GP, grass pollen.

*Baseline FEV₁ measured on the allergen study day.

†FEV₁ measured on the methacholine study day.

variations in histamine levels.^{2, 4} This is not paralleled by an increase in prostaglandin D₂,⁴ which characterizes the early mast cell-dependent airway response to allergen, and thus cannot be accounted for by mast cell degranulation.

Basophils demonstrate SHR ex vivo that is exaggerated in asthma.^{5, 6} Since basophils account for >98% of total blood histamine and there is significant correlation between the level of histamine in plasma and total blood,⁶ it has been suggested that the basophil is the major determinant of resting plasma histamine levels. Consistent with the enhanced histamine releasability from these cells, the histamine levels in plasma are higher in subjects with asthma than in normal control subjects.⁷ It is thus possible that the late elevations in plasma histamine detected during the LAR⁸ and attributed to mast cell degranulation relate, instead, to an alteration in basophil histamine releasability.

To examine whether SHR from human basophils is altered after experimental allergen inhalation, we have challenged six subjects with atopic asthma with allergen to induce a dual (EAR and LAR) asthmatic reaction. During a period of 8 hours after challenge, the magnitude of the asthmatic reaction was recorded by spirometry, and venous blood was obtained for measurement of basophil SHR and plasma histamine levels. To provide control for any effects of bronchoconstriction per se and diurnal variation on basophil releasability, two additional challenges were undertaken on separate occasions: one challenge with methacholine to cause the same degree of bronchoconstriction achieved during the EAR to allergen, and one challenge with physiologic saline.

MATERIAL AND METHODS

Subjects

Six male subjects with atopic asthma (mean age, 23.7 years; range, 19 to 30 years) with perennial asthma of vary-

ing severity participated in the study (Table I). Methacholine challenge demonstrated a range of airway hyperresponsiveness, as demonstrated by PC₂₀ methacholine (geometric mean PC₂₀, 0.78 mg/ml; range, 0.13 to 6.28 mg/ml). All subjects had positive skin prick tests to at least two of seven common inhalant allergens. Four of six subjects had elevated total serum IgE levels, and all subjects had elevated IgE specific for the allergen used for bronchial challenge. None were smokers and none had suffered from respiratory infections for at least 2 months before the study. Their therapy consisted of regular inhaled β_2 -agonists (all subjects) and inhaled beclomethasone dipropionate (one subject). Before the study, all subjects developed dual asthmatic responses after allergen bronchial challenge consisting of an EAR, reaching a maximum between 15 and 30 minutes after inhalation, and an LAR, defined as a >15% fall in FEV₁, developing between 4 and 8 hours after challenge.

Study design

At least 2 weeks before the beginning of the study, PC₂₀ methacholine was determined, and allergen bronchial challenge was performed to determine the concentration of allergen causing a fall in FEV₁ >20% and <40% from baseline (PC_{>20} allergen) and to ensure that all subjects were dual asthmatic responders. Subsequently, the subjects attended on three occasions at an interval of 2 to 3 weeks for an allergen study day, a methacholine study day, and a physiologic-saline study day. Inhalation challenge with PC_{>20} allergen was performed on the first visit with measurements of FEV₁ and venous blood sampling before challenge (baseline) and 2, 5, 10, 15, 60, 120, 240, 360, and 480 minutes after challenge. On the second visit, the same procedure was repeated except that the challenge was performed with a single challenge dose of methacholine causing a >20% fall in FEV₁ (PC_{>20} methacholine) determined on the visit when PC₂₀ methacholine was established. On the final, third visit, four subjects underwent a sham challenge with physiologic saline, and blood was obtained before challenge and at 120, 240, 360, and 480 minutes after challenge for the measurement of SHR only. On all three study days, subjects had stopped their β -agonist therapy for at least 8

hours and their inhaled corticosteroids for 48 hours before challenge. All challenges were performed at the same time of day, between 9 and 10 AM.

Atopic status

Skin prick testing was performed with seven common inhalant allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, mixed grass pollen, cat fur, dog hair, feathers, and a mixture of molds, Bencard, Brentford, Middlesex, U.K.). The tests were accepted as positive if the skin wheal diameter was 3 mm larger than that of the saline control wheal. On the day of the allergen challenge, blood was obtained and stored for subsequent analysis of total serum IgE by ELISA. IgE specific for the same allergen extracts used for bronchial challenge was assayed with RAST. For the latter assay, serum was incubated with allergen-coupled disks in flat-bottom, 96-well microtiter plates and incubated for 3 hours, washed, and further incubated with ^{125}I -labeled rabbit antihuman IgE antibody. The counts per minute obtained from known negative human serum samples were measured and subtracted from the mean counts of each subject with asthma. All counts were performed in triplicate.

Bronchial challenge

The allergen and methacholine challenges were performed with a modified method according to Chai et al.⁹ The aerosolized solutions were administered via an Inspiron nebulizer (CR Bard International Ltd, Sunderland, U.K.) driven by compressed air at a flow of 8 L/min that produces a mass median diameter of 4.7 μm and has a delivery rate of 0.33 ml/min. FEV₁ was measured with a dry-wedge spirometer (Vitalograph Ltd., Buckingham, U.K.).

On the day when airway responsiveness to methacholine was determined, five breaths of physiologic saline followed by increasing concentrations of methacholine (Sigma Chemical Co. Ltd., Poole, U.K.), ranging from 0.03 to 8.00 mg/ml, were inhaled from functional residual capacity to total lung capacity. The best of two readings at 1 and 3 minutes after inhalation was recorded at each time point, and the lower of the two values was taken. The inhalations were continued until the FEV₁ had fallen by >20% from the postsaline baseline, and the percentage fall was plotted against the logarithmically transformed cumulative concentration of inhaled methacholine. The PC₂₀ methacholine was calculated by linear interpolation between the last two points of the dose-response curve. The highest concentration, which had caused a >20% fall in FEV₁ on that occasion, was taken as the PC_{>20} methacholine.

During allergen challenge to establish PC_{>20} allergen, five breaths of fivefold to tenfold increasing concentrations of allergen (Bencard Ltd.) were administered, and single measurements of FEV₁ were taken at 5, 10, and 15 minutes after inhalation of each solution until a >20% fall in FEV₁ had occurred. The allergens used for bronchial challenge are presented in Table I, selected as that allergen eliciting the largest skin wheal reaction.

On the three study days, when blood was obtained to

study basophil function, challenges with PC_{>20} allergen, PC_{>20} methacholine, and physiologic saline were performed with a single challenge consisting of five breaths of the respective solutions taken from functional residual capacity to total lung capacity.

Measurement of plasma histamine and ex vivo basophil histamine releasability

The following materials were purchased: Dextran 110 and heparin, 5000 U/ml, CP Pharmaceutical Ltd. and Sigma Chemical Co. Ltd.; HEPES, sodium chloride, glucose, butanol, heptane, hydrochloric acid, orthophosphoric acid, ethylenediaminetetraacetic acid, sodium hydroxide, and Triton X-100 (BDH Chemicals, Poole, U.K.). HBSG was made of 4 gm of NaCl, 0.5 gm of glucose, 2.4 gm of HEPES, 0.5 ml of KCl (2.7 mol/L), 0.5 ml of NaH₂PO₄ (0.4 mol/L), and 500 ml of distilled H₂O; HBSG-Ca⁺⁺/Mg⁺⁺ solution was made of HBSG with 0.5 ml of CaCl₂ (1.8 mol/L) and 0.5 ml of MgCl₂ (0.5 mol/L). The final pH of the solutions was adjusted to 7.2 with 50% NaOH.

A 16-gauge cannula (Critikon, Ascot, U.K.) was placed in a vein of the antecubital fossa and kept patent by flushing with physiologic saline. After a 30-minute rest, baseline measurements of FEV₁ were made and two samples of blood were obtained at 10-minute intervals. The subjects then inhaled five breaths of either PC_{>20} allergen, PC_{>20} methacholine, or physiologic saline, and blood was obtained at the set time intervals with the first 1.5 ml of blood being discarded and 25 ml collected.

For the measurement of plasma histamine, 15 ml of blood was immediately transferred into a tube containing 150 μl of ethylenediamine tetraacetic acid (0.5 mol/L) to inhibit diamine-oxidase activity and rapidly cooled to 4° C on ice; the plasma was separated within 30 minutes by centrifugation at 2700g for 12 minutes at 4° C. To avoid basophil contamination from the underlying leukocyte coat, only the top 2 ml of plasma was aspirated for histamine assay and stored at -80° C before histamine assay. Plasma histamine was quantified in duplicate by RIA (Serotec, Marcellles, France) whose sensitivity was 0.2 ng/ml.

To measure ex vivo basophil HR, 10 ml of blood was transferred into 1.6 ml of solution comprised of 100 ml of dextran, 4.5 gm of glucose, and 1.25 ml of heparin (5000 U/ml) and allowed to sediment for 90 minutes. The buffy coat was separated and pelleted by centrifugation at 170g at 4° C for 5 minutes, and the cell pellet was resuspended in ice-cold HBSG. The cells were repelleted by centrifugation at 170g at 4° C for 5 minutes and resuspended in 3 ml of HBSG-Ca⁺⁺/Mg⁺⁺; 0.5 ml aliquots were added to tubes containing 0.5 ml of HBSG-Ca⁺⁺/Mg⁺⁺. An additional 0.5 ml aliquot was kept for measurement of total cell histamine. All assays were performed in duplicate. The cell suspensions were incubated for 45 minutes at 37° C with occasional gentle mixing, after which 100 μl of 5% trichloroacetic acid was added for cell lysis to the tubes designated for total cell histamine analysis. All tubes were then centrifuged at 170g at 4° C for 5 minutes, and the super-

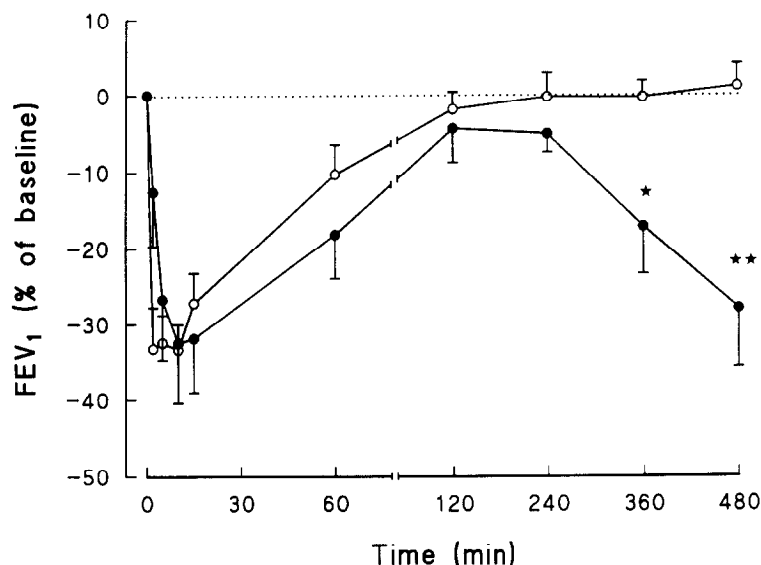


FIG. 1. Mean changes in FEV₁ from baseline values on allergen (●) and methacholine (○) study days during a period of 8 hours after challenge. Vertical bars, SD (* $p < 0.01$ and ** $p < 0.001$, difference in FEV₁ percent of baseline between allergen and methacholine study days).

natants were aspirated. One hundred microliters of 5% trichloroacetic acid was added to each supernatant designated for analysis of spontaneous release. The supernatants were stored at -80°C for subsequent histamine analysis, which was performed by automated fluorimetry.

The results of the HR studies are presented as the net amount of histamine released into the supernatant expressed as a percentage of total cell histamine.

Statistical analysis

The distribution of FEV₁ values was assumed to be normal. The data from HR and plasma histamine assays demonstrated large variation and were assumed not to be normally distributed. The measured values of FEV₁ were plotted against time, and the time-response AUC was calculated by trapezoid integration between 0 and 15 minutes after challenge for the EAR and between 4 and 8 hours after challenge for the LAR. The Friedman's test was used first to determine differences between the values of plasma histamine and basophil HR at the various time points to avoid obtaining significant differences because of the use of multiple Wilcoxon's tests. Only if this test identified significant differences was the Wilcoxon's signed-rank test used to determine differences in HR and plasma histamine between any individual time point and baseline values and between the same time points on the allergen and methacholine study days. The Student's t test for paired data was used for comparisons of FEV₁ on the two days. Results were accepted as being statistically significant if p was <0.05 .

RESULTS

The levels of total and specific serum IgE and values of PC_{>20} allergen and PC_{>20} methacholine used for challenge are presented in Table I.

Measurements of FEV₁

There was no significant difference in the mean $\pm$ SEM baseline FEV₁, represented as a percentage of predicted, between the allergen ($99.2\% \pm 7.5\%$) and methacholine ($100.5\% \pm 8.3\%$) study days ($p > 0.05$) (Table I). All the subjects developed an EAR after both allergen and methacholine challenge and an LAR on the allergen study day (Fig. 1). Four subjects who underwent a sham challenge with physiologic saline maintained their FEV₁ within 5% of baseline throughout the period of observation (data not presented).

The maximum fall in FEV₁ from baseline during the EAR on the allergen study day ($32.5\% \pm 7.9\%$) was not significantly different from that on the methacholine study day ($33.4\% \pm 3.4\%$), nor was there any difference between the time-fall in FEV₁ AUC values ($p > 0.05$). On the allergen study day, FEV₁ gradually returned to within $4.4\% \pm 3.7\%$ of baseline values by 120 minutes ($p > 0.05$) and was followed by a further decline to reach a maximum $28\% \pm 7.7\%$ fall in FEV₁ from baseline at 8 hours after challenge ($p < 0.001$). On the methacholine study day, no LAR was observed, and the AUCs of the 4- to 8-hour periods on the 2 study days were significantly different ($p < 0.001$).

Spontaneous ex vivo HR

The individual subject data of changes in spontaneous HR after allergen, methacholine, and physiologic saline challenge are illustrated in Fig. 2, and data of group median values are illustrated in Fig. 3.

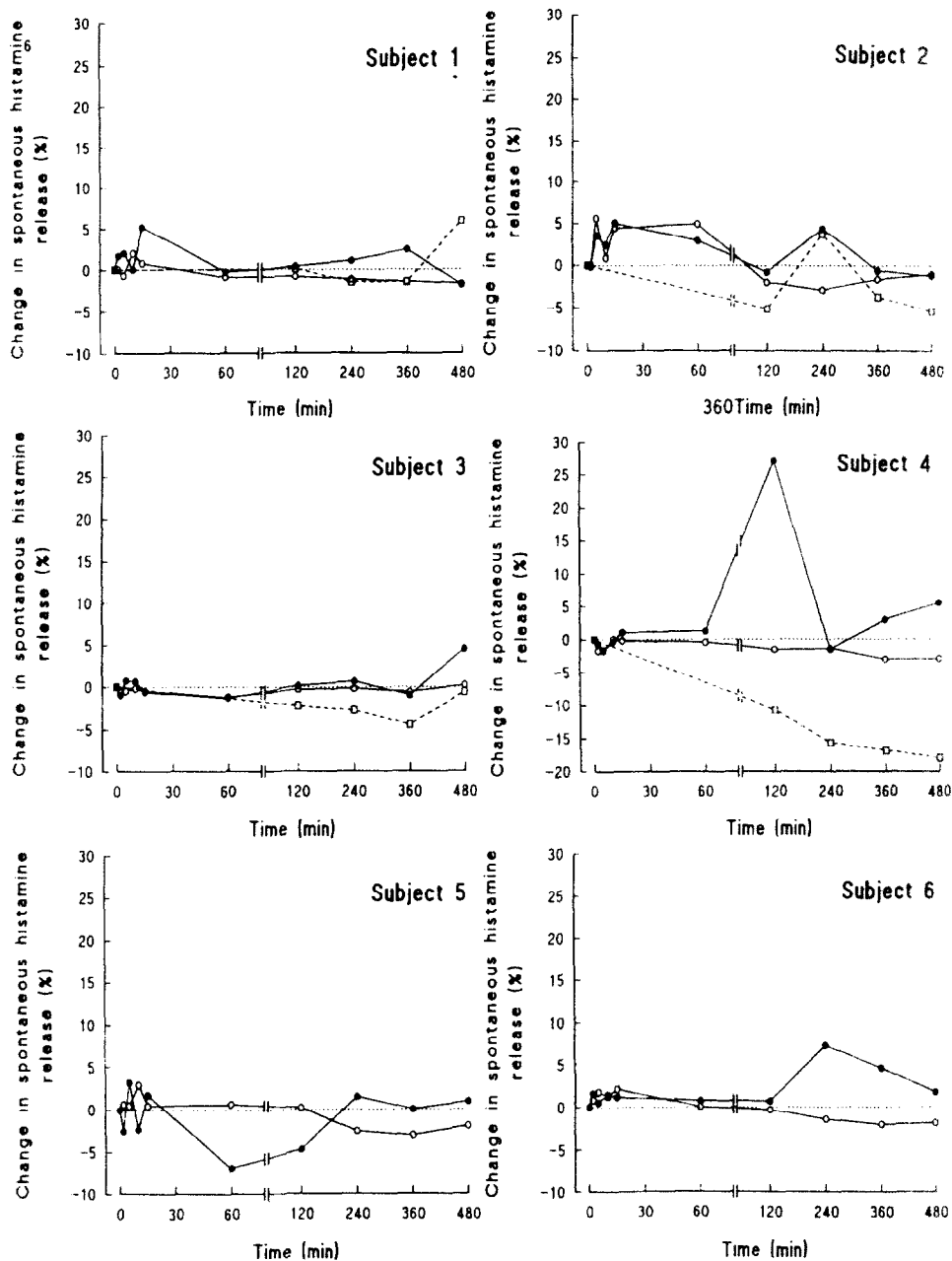


FIG. 2. Absolute changes in percent SHR in individual subjects after allergen (●) (N = 6), methacholine (○) (N = 6), and physiologic saline (□) (N = 4) inhalation challenge.

There was no significant difference in basal SHR on the allergen (median, 4.1%; range, 1.4% to 19.1%) and methacholine (median, 4.6%; range, 3.9% to 7.2%) study days ($p > 0.05$). Median HR on the saline study day was higher (median, 8.9%; range, 2.9% to 26.3%) than on both allergen and methacholine study days, but these differences were not compared for statistical significance because of small numbers of subjects studied on the physiologic saline study day.

Friedman's test demonstrated that SHR after both allergen and methacholine challenge departed significantly from baseline. SHR rose significantly ($p < 0.05$) after allergen challenge during the EAR, reaching a maximum at 15 minutes (median absolute change in histamine release of 1.5%; range, -0.7% to 5.2%) (Fig. 3). SHR returned toward baseline at 1 hour but was followed by a further rise at 4 hours, reaching a maximum at 8 hours (median absolute change of 1.3%; range, -1.8% to 5.6%). This late

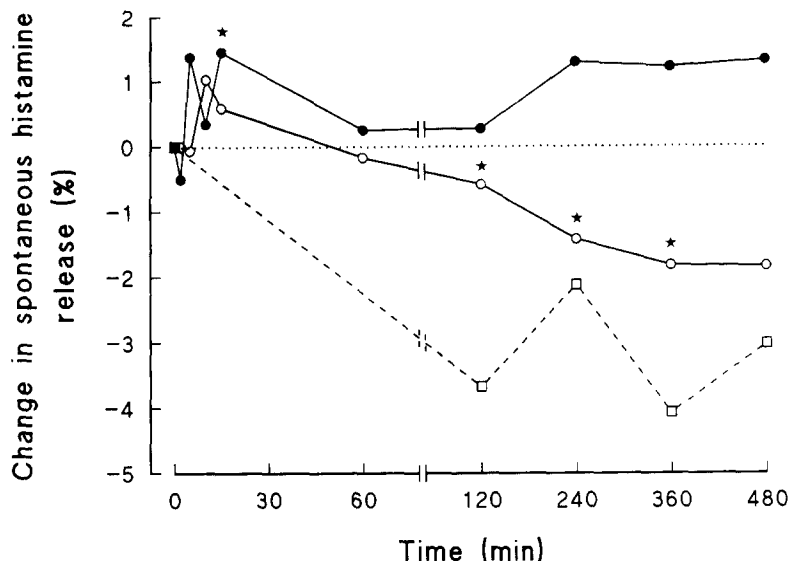


FIG. 3. Absolute changes in percent SHR after allergen (●) (N = 6), methacholine (○) (N = 6), and physiologic saline (□) (N = 4) inhalation challenge, illustrated as median group values (* $p < 0.05$ by comparison with baseline).

change, however, was not significantly higher than at baseline ($p > 0.05$). On the methacholine study day, SHR decreased beyond baseline between 2 and 8 hours, reaching a nadir at 6 hours (median absolute change of -1.8% ; range, -3.1% to -0.6% , when compared with that at baseline, $p < 0.05$ (Fig. 3). The difference in change in SHR between the allergen and methacholine study days was significant at 2, 4, and 6 hours after challenge ($p < 0.05$).

After inhalation of physiologic saline, there was a decrease in SHR similar to that observed on the methacholine study day (median absolute decrease of -3.0% ; range, -18.0% to 5.9% at 8 hours, Fig. 3). Because of small numbers of subjects (N = 4) in this part of the study, these data were not compared by statistical tests with data obtained after allergen and methacholine challenge.

Plasma histamine

The median baseline plasma concentration of histamine was 0.36 ng/ml (range, 0.13 to 0.44 ng/ml) on the allergen study day, which was not significantly different from that measured on the methacholine study day (median, 0.38 ng/ml; range, 0.27 to 0.56 ng/ml) with no significant change from baseline on either study day ($p > 0.05$) (Fig. 4).

DISCUSSION

With standard bronchial challenge to induce a dual asthmatic reaction, we have demonstrated in the present study that allergen inhalation causes a small, but

significant, increase in SHR by circulating basophils. This increase occurred in parallel with the bronchoconstrictor response of the EAR and could not be explained by bronchoconstriction alone since it was not reproduced by methacholine challenge leading to a similar degree of fall in FEV₁. SHR remained elevated during the 8 hours after inhalation of allergen, whereas HR decreased after inhalation of both methacholine and physiologic saline, suggesting that inhalation of allergen by sensitive individuals modifies basophil function.

Consistent with our results, several investigators have reported increased SHR by circulating basophils in atopic asthma. This phenomenon appears to be a genetically transmitted,¹⁰ "intrinsic" abnormality of the cell that is unrelated to serum levels of IgE.^{5, 11, 12} Concordant with this finding, we have been unable to identify any relationship between SHR and total IgE in the subjects in the present study. We have extended this observation by demonstrating that this function of basophils also fails to correlate with the level of IgE specific for the same allergens used during bronchial challenge.

Although little is known about the effects of allergen on spontaneous and stimulus-induced basophil releasability, in vitro exposure of basophils to subthreshold concentrations of allergen, which are alone insufficient to cause mediator release, has been demonstrated to sensitize these cells to subsequent stimulation with deuterium oxide.¹¹ In addition, natural allergen exposure in pollen-sensitive subjects with

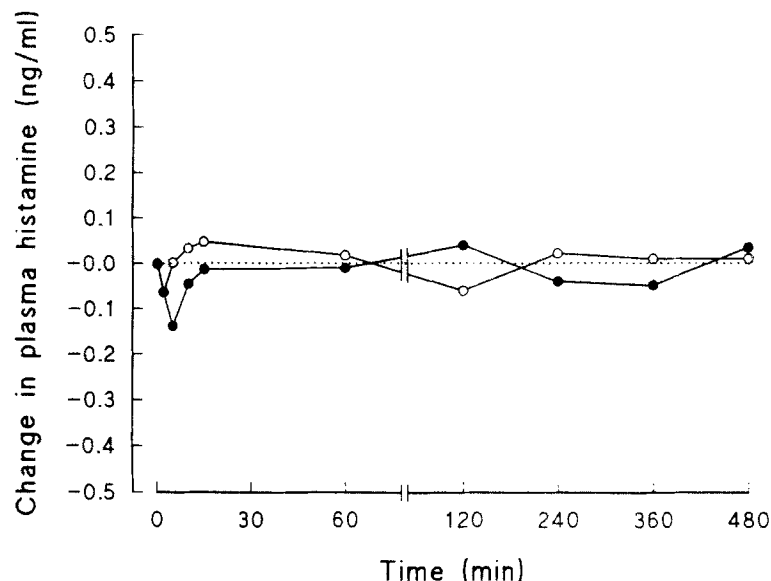


FIG. 4. Median changes in plasma histamine levels after allergen (●) and methacholine (○) challenge (N = 6).

seasonal asthma is associated with increased spontaneous and allergen-stimulated production of HRFs,¹³ which correlates with the seasonal change in airway responsiveness. The immediate effects of allergen on SHR could thus reflect either direct contact with basophils or an indirect action via HRFs or cytokines, such as IL-3, released from other cell types after allergen stimulation. However, the interaction between allergen and circulating basophils in our study remains to be elucidated. Passage of allergen from the airways into the blood stream is a possibility, since as few as 100 molecules of antigen per cell are capable of initiating HR.¹⁴ Potentially, a direct effect of allergen on basophil function would be to either increase SHR or to prime the cell for additional stimuli. It is, however, unlikely that persistent allergen stimulation could solely account for the late differences in SHR identified on the allergen study and control study days. A more likely explanation for the LAR-associated enhanced basophil releasability is activation of this cell subsequent to the effects of HRFs¹⁵⁻¹⁸ and cytokines, such as granulocyte-macrophage-colony-stimulating factor, IL-3,^{19, 20} and IL-1,²¹ produced by inflammatory cells present in the airways. The capacity of allergen to modulate HR has been demonstrated in studies of seasonal asthma demonstrating an increase in spontaneous production of mononuclear cell-derived HRFs.¹³ Allergen-related cytokine production has also been suggested in studies demonstrating increased circulating basophil-eosinophil progenitors during natural²² and experimental²³ exposure to allergen, whereas bronchoalveolar lavage in subjects with

atopic asthma has demonstrated elevated levels of granulocyte-macrophage-colony-stimulating factor, and IL-1 β .²⁴ An interaction between IgE-dependent mechanisms and cytokine stimulation has been suggested by a recent study demonstrating that subcutaneous administration of recombinant human IL-3 enhances HR from basophils stimulated with anti-IgE.²⁵

In the present study, plasma histamine was demonstrated not to increase after allergen challenge despite the increase in basophil releasability. However, it is unclear how spontaneous basophil releasability, which is measured *ex vivo*, reflects *in vivo* mediator release. Current methods of measurement of histamine releasability have demonstrated SHR to be as high as 80% in various allergic disorders.⁵ It is clear that such high releasability could not be operating *in vivo* and that the high values reflect a general basophil hyperactivity that renders these cells more sensitive to the extensive manipulation during experiments that are performed in an environment unsuitable to cells. Thus, the changes in basophil release in our study do not necessarily reflect significant changes in measurable *in vivo* mediator release but more a change in basophil activity. In addition, patient selection may be an important determinant of the ability to detect late changes in plasma histamine levels. To date, this has only been demonstrated in the EAR and depends on the level of sensitization and airway responsiveness.²⁶ The degree of airway responsiveness limits the dose of allergen that may be delivered to induce an EAR, and it is conceivable that basophil responses to stimulation may also be dose dependent during the

LAR. The resting level of histamine in plasma in the subjects of this study (median, 0.36 ng/ml) is similar to level identified in previous studies that have failed to demonstrate increments in plasma histamine after allergen bronchial challenge.²⁶ The failure to detect plasma histamine changes during the EAR in these subjects has been interpreted as indicative of a lesser degranulation of airway metachromatic cells than in those subjects with asthma with lower resting plasma histamine and airway responsiveness in whom changes can be identified after challenge. The one study that has demonstrated late changes in plasma histamine after allergen inhalation was in subjects with lower resting plasma histamine levels who, by inference, received a larger allergen dose.⁸ An additional factor may be related to the IgE status of the selected subjects with asthma. Although no relationship exists between IgE levels and SHR, the ability to modulate HR by factors, such as IL-3, has been closely related to the presence of the so-called IgE⁺ antibodies²⁷ present in a subpopulation of atopic subjects whose HR is also sensitive to deuterium oxide.²⁸ However, in this study, we have not attempted to demonstrate the presence of IgE⁺ antibodies. Finally, any increase in HR resulting from allergen inhalation may have been restricted by the homeostatic metabolic mechanisms of histaminase, such as diamine oxidase, thus annulling the effect of HR in the plasma.

The results of this study add additional evidence of inflammatory cell activation in atopic asthma. Leukocyte activation, as demonstrated by changes in neutrophil chemotactic activity and formation of neutrophil and complement rosettes in parallel with changes in FEV₁, which occur after allergen bronchial challenge in dual, but not single, asthmatic responses,²⁹ suggests that this phenomenon may be a feature of sustained airway inflammation of the LAR. Recently, evidence has been provided of T-lymphocyte activation in both peripheral blood in acute exacerbations of asthma³⁰ and in bronchoalveolar lavage of subjects with atopic asthma with mild to moderate disease severity.³¹ Furthermore, immunohistochemistry has demonstrated activated T-lymphocytes in the airway mucosa of subjects with atopic asthma.³² Our study now suggests that allergen challenge has an effect on basophil activity and abolishes its diurnal change in SHR. All these changes in peripheral leukocyte function can be explained by cytokine release from activated airway cells, although further studies will be needed to elucidate the precise mechanisms and also to identify whether more marked increases in HR could be induced in a subpopulation of subjects with atopic asthma possessing IgE⁺ antibodies.

In conclusion, we have demonstrated enhanced

spontaneous basophil releasability after allergen challenge in subjects with asthma who have developed an LAR after allergen inhalation. The nature of the allergen-induced factors that determine histamine releasability remains to be determined but could involve HRFs or other unrelated cytokines that would be produced by inflammatory cells present in the airways. This study provides further support for cell activation by allergen in atopic asthma.

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