

Allergic bronchial asthma: eosinophil chemotaxis and antihistaminic drug modulation

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This paper investigated the chemotactic capacity of blood eosinophils (EOS) in response to C5a and formyl-leucyl-phenylalanine (FMLP) in 15 *Parietaria*-pollen-allergic patients with mild asthma during the *Parietaria* pollen season. Data showed that EOS chemotaxis toward C5a was significantly reduced during the peak pollen count, compared with the values obtained in normal subjects. On the other hand, FMLP could induce EOS chemotactic activity only in *Parietaria*-allergic patients, the highest value being registered during the height of the *Parietaria* season. *In vitro* cimetidine modulation of chemotactic function in comparison with C5a led to a recovery of the impaired results obtained in the presence of high histamine levels. Moreover, diphenhydramine preincubation of EOS induced a reduction of the enhanced chemotactic activity obtained with low concentrations. The data suggest that different serum histamine levels give rise to an impaired immune-phlogistic response in allergic patients and that histamine-receptor antagonists may modulate this effect.

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Various cells, such as eosinophils (EOS), are known to participate in the inflammatory events observed during the allergen-induced, late-phase reaction of allergic bronchial asthma. Indeed, several authors have reported an increased number of EOS in the blood of allergic patients (1–3). It is well known that EOS can generate a wide range of mediators (4–7) and that their ability to induce infiltration and inflammation of the lung tissue is essential to the development of the allergen-induced, late-phase asthmatic reaction (1, 8). Moreover, the infiltration of EOS in lung tissue seems to correlate with the severity of airways hyperresponsiveness and the pathologic features of asthma (9).

However, the mechanisms by which EOS migrate to the inflammatory sites remain unclear. It has been postulated that the expression of specific adhesion molecules (10) and “priming” induced by lymphokines, such as interleukin (IL)-3, IL-5, and granulocyte/macrophage colony-stimulating factor (GM-CSF) may play a key role (11). Indeed, Warringa et al. (12) have elegantly demonstrated that preincubation of EOS with low doses of GM-CSF or IL-3 leads to

a marked chemotactic response of these cells to the neutrophil chemotaxins NAF/IL-8 and formyl-leucyl-phenylalanine (FMLP), and modulates the responses to platelet-activating factor (PAF), leukotriene (LT)_{B₄}, and C5a, potent chemotaxins for EOS. Moreover, it has been shown that serum histamine levels may modulate this immunopathologic event (13, 14). In this regard, we have previously reported that a high concentration of histamine led to a significant reduction of EOS and polymorphonuclear cell (PMN) chemotactic responsiveness, while a low concentration led to an increase of chemotaxis. These effects were reversed by preincubation of cells with cimetidine and diphenhydramine, which abrogated, respectively, the effects mediated by high and low histamine levels (15).

Thus, to clarify the mechanisms by which EOS accumulate and persist at allergic inflammatory sites, we have evaluated, in the present study of patients with mild *Parietaria*-pollen-allergic asthma, the chemotactic response of EOS to FMLP and C5a during an entire year. Moreover, the *in vitro* effect of cimetidine and diphenhydramine,

antagonists of H₂ and H₁ receptors, respectively, on EOS responsiveness was also evaluated.

Material and methods

Patients

Fifteen *Parietaria*-pollen-allergic patients with mild asthma were examined three times before, five times during, and three times after the pollen season. There were nine males and six females (mean age 32 years, range 15–52 years). Patients had a history of rhinoconjunctivitis and wheezing during the *Parietaria*-pollen season, positive skin prick test and RAST, presence of significant airway obstruction (FEV₁<70% predicted), and β₂-adrenergic bronchodilator aerosol reversibility (at least 20%). Most of them (12 patients) had a positive family history of atopic diseases, and three were average smokers. None of the patients had taken any form of corticosteroid drug, undergone immunotherapy, or been affected by respiratory tract infection before this study. Twenty healthy donors with no history of allergy or asthma were considered controls (mean age 29 years, range 20–51 years).

Chemicals

Histamine and diphenhydramine were purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA). Cimetidine was generously donated by Smith Kline and French Laboratories, Hertfordshire, UK.

Eosinophil isolation

The EOS were obtained from peripheral blood according to the method of Sher & Glover (16). Briefly, after PMN isolation with 6% dextran (MW 40 000) (Pharmacia, Sweden), the cells were washed thrice in phosphate-buffered saline (PBS) (pH 7.5, 0.2 M), resuspended in 9.5 ml of PBS, and supplemented with carbonyl iron (General Airline and Film Corporation, Linden, NJ, USA). After incubation for 10 min at 37°C under mild shaking, the cells were placed on ice for a few minutes. Then, cells with ingested carbonyl iron particles were removed with a magnet. The purity of the EOS population ranged from 85% to 95% as detected by staining with yellow eosin by the method of Durham & Kay (1).

Histamine, cimetidine, and diphenhydramine pretreatment

After centrifugation, EOS were resuspended in time-point-matched autologous serum containing

either 10⁻³ M cimetidine or diphenhydramine for 1 h at 37°C, and then washed thrice in PBS and used for the final experiments reported in Table 1. We used a dose of 10⁻³ M for both histamine-receptor antagonists because, in a pilot dose-response curve (10⁻²–10⁻⁸ M), it proved to be the most efficient and least toxic dose, as assessed by the trypan blue exclusion test and lactate dehydrogenase assay (15). In some experiments, normal EOS were incubated in PBS containing either histamine at 10⁻² or 10⁻⁶ M with or without 10⁻³ M of both histamine-receptor antagonists (Table 2).

Chemotaxis

Chemotaxis was performed by a modified Boyden chamber technique, as described by Snyderman et al. (17, 18). Then, 0.2 ml of cell suspension was placed in the upper compartment of a Boyden chamber (Neuroprobe, Bethesda, MD, USA), and 0.2 ml of the chemotactic factor was used to fill the lower compartment. A 5-μm pore, about 120-μm-

Table 1. Cimetidine and diphenhydramine modulation of eosinophil chemotactic response to C5a in *Parietaria*-allergic patients during 1 year

Subjects	No. of subjects	Treatment	Chemotactic index (μm) (mean ± SD)	
Healthy donors	20	None	15.9 ± 3.6	
		Cimetidine	17.4 ± 3.7*	
		Diphenhydramine	12.1 ± 2.1*	
Allergic subjects	15	At peak pollen count	None	7.2 ± 2.5
		Cimetidine	17.2 ± 4.5**	
		Diphenhydramine	5.4 ± 3.1*	
	After season	None	18.4 ± 4.2	
		Cimetidine	19.8 ± 3.3*	
		Diphenhydramine	10.1 ± 2.8**	

* Not significant in comparison with homologous untreated cells; ** P<0.001 in comparison with homologous untreated cells.

Table 2. *In vitro* cimetidine and diphenhydramine modulation of normal eosinophil chemotactic response to C5a after high (10⁻² M) and low (10⁻⁶ M) histamine concentrations

Cells	Treatment	Chemotactic index (μm) (mean ± SD: 15 values)
EOS	None	10.6 ± 3.2
EOS (pretreated with 10 ⁻² M histamine)	None	3.3 ± 2.1
	Cimetidine 10 ⁻³ M	15.6 ± 3.4*
EOS (pretreated with 10 ⁻⁶ M histamine)	None	18.4 ± 2.3
	Diphenhydramine 10 ⁻³ M	11.2 ± 3.3*

* P<0.001 in comparison with homologous histamine-pretreated cells.

thick polycarbonate filter separated the chamber compartments. A cleavage product of the fifth complement component obtained by incubating fresh AB serum with zymosan A (Sigma Chemical Co., St Louis, MO, USA) (18) was used as stimulant. In another set of experiments, FMLP was tested as chemotactic agent. FMLP was purchased from Sigma Chemical Co, St Louis, MO, USA. Buffer alone was used as control. From previous reports (11), and from our pilot dose-response curves (data not shown), 10 nmol/l proved to be the optimal concentration for both chemoattractants to induce EOS migration. The chambers were incubated for 90 min at 37°C in humidified air containing 5% CO₂. Thereafter, the filters were removed, washed in saline, fixed in ethanol, and mounted on glass slides. The cells were then counted, at every 10-µm interval from the original monolayer to the distal surface, in 5–10 high-powered fields (hpF), and the results were expressed as the average distance (µm) migrated by the cells in 90 min of incubation (19).

Statistical analysis

Differences in chemotactic response within groups were statistically evaluated with Student's *t*-test for paired observations, and differences between normal donors and allergic patients with Student's *t*-test for unpaired observations. Differences with *P* values of less than 0.05 were considered statistically significant.

Results

Fig. 1 demonstrates that, before and after the *Parietaria*-pollen season, C5a-triggered chemotactic activity of EOS derived from the circulation of allergic patients was similar to that observed in healthy donors. In contrast, during the *Parietaria*-pollen season, EOS migration was significantly reduced in allergic patients compared to healthy donors, reaching the minimum value during the maximal pollen count ($P < 0.001$). Thus, while EOS from healthy donors showed a flat chemotactic index in response to C5a during the entire year, an upside-down bell-shaped profile was observed for EOS belonging to allergic patients. Moreover, different patterns were observed when FMLP was used as chemotaxin (Fig. 2). Indeed, EOS from healthy donors were unable to respond to FMLP, while the chemotactic responsiveness of EOS from allergic subjects, to the same chemotaxin, was significantly increased during the entire year, reaching the higher value at the maximal pollen count.

As shown in Table 1, EOS from allergic patients preincubated with cimetidine were able to recover the reduced chemotactic capacity observed during the period of maximal pollen count. However, cimetidine did not influence the EOS chemotactic capacity observed in healthy donors, as well as in allergic subjects, both after (Table 1) and before the season (data not shown). Interestingly, diphenhydramine induced a statistically significant

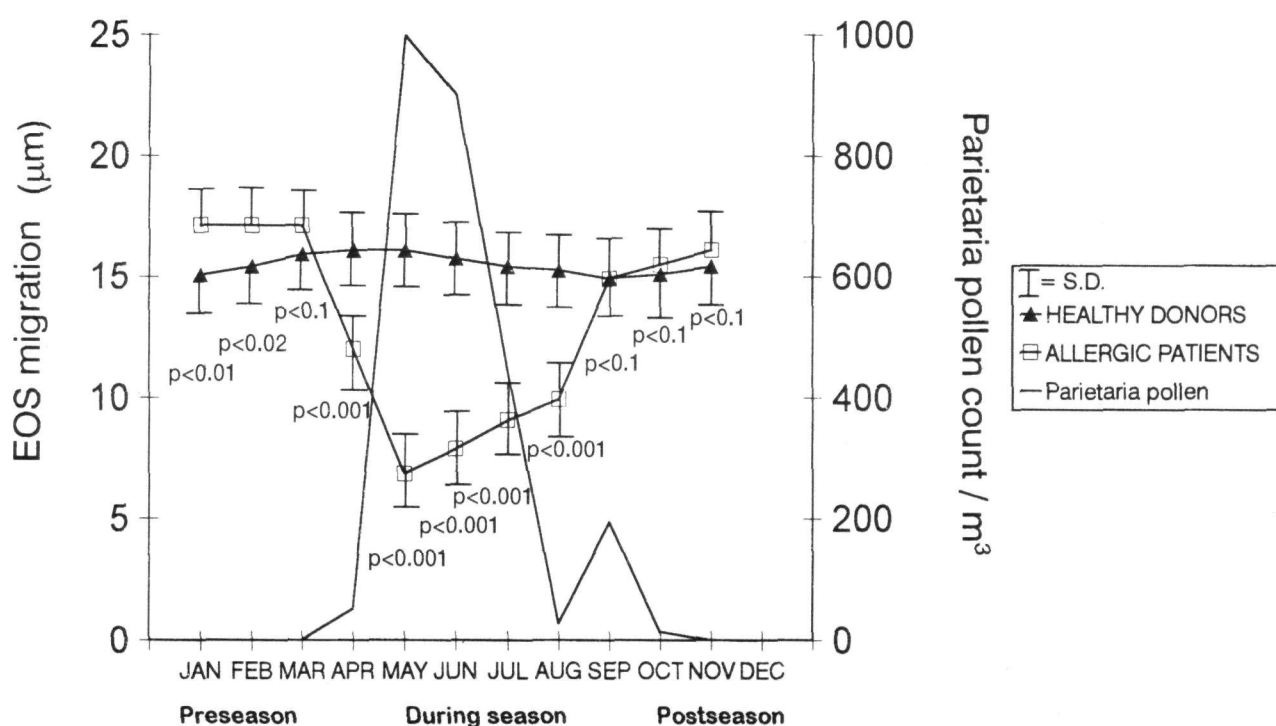


Fig. 1. C5a-triggered chemotactic activity in allergic patients and healthy donors. EOS chemotactic response to C5a evaluated in both 20 healthy donors and 15 *Parietaria*-allergic patients 11 times during 1 year.

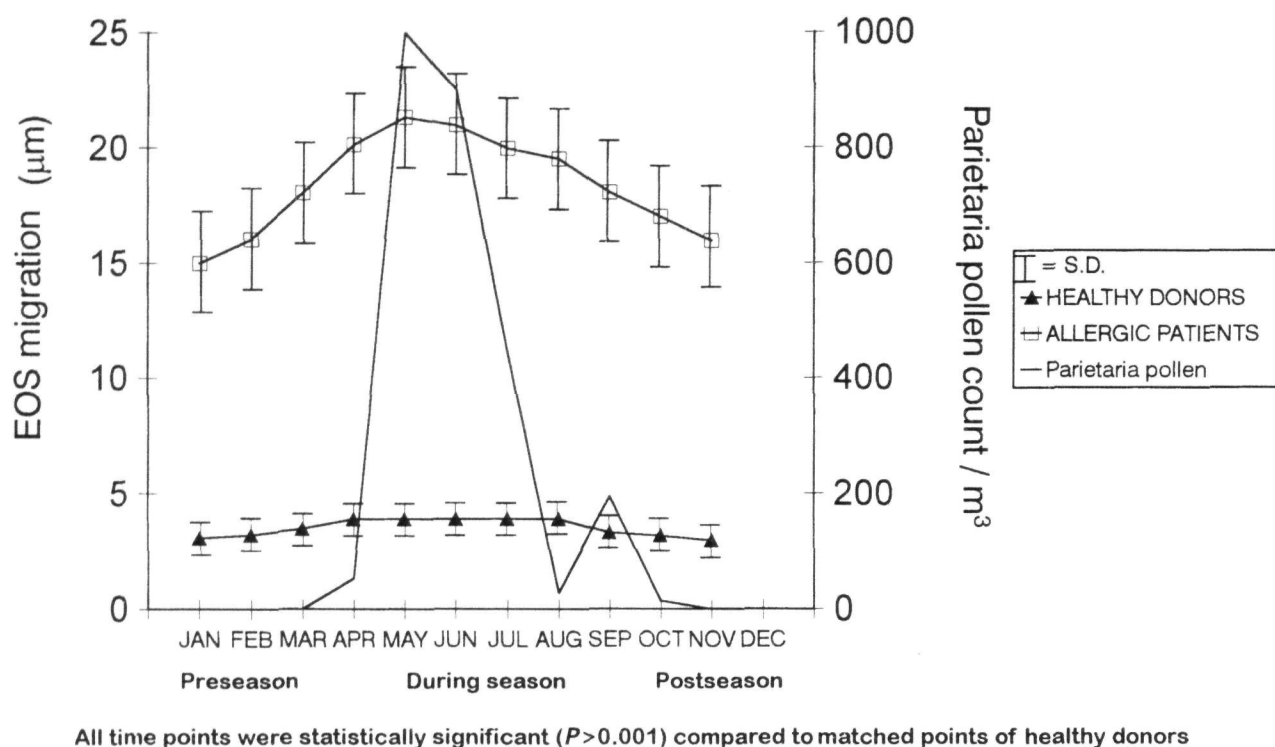


Fig. 2. FMLP-triggered chemotactic activity in allergic patients and healthy donors. EOS chemotactic response to FMLP evaluated in both 20 healthy donors and 15 *Parietaria*-allergic patients 11 times during 1 year.

reduction of the EOS chemotactic capacity of allergic patients after the season, but not at the peak pollen count (Table 1). Indeed, no statistically significant differences were observed in EOS chemotactic activity between homologous untreated and diphenhydramine-treated cells in both healthy donors and allergic subjects at the peak pollen count (Table 1). Following the same experimental conditions, the results obtained before the season were comparable to those observed after it (data not shown).

For study of the effect of histamine on EOS chemotactic capacity, EOS from normal subjects were incubated in PBS with 10^{-2} and 10^{-6} M of histamine with or without 10^{-3} M of either cimetidine or diphenhydramine. The doses of 10^{-2} and 10^{-6} M of histamine were chosen on the basis of a pilot study (15). As shown in Table 2, cimetidine was able to enhance the C5a-induced chemotaxis suppressed by high histamine concentration (10^{-2} M), while diphenhydramine reduced the higher C5a-induced chemotaxis observed with low histamine level (10^{-6} M).

Discussion

It is universally recognized that in the late-phase asthmatic reaction T cells and EOS play an important role in the pathogenesis of allergic asthma and other allergic diseases (2, 3). Thus, in the present study, we focused on EOS chemotactic capacity, in healthy and allergic subjects. EOS isolated from normal subjects, as previously reported, were unresponsive to FMLP but quite responsive to C5a (20). Interestingly, EOS isolated

from the blood of allergic subjects proved to be "primed" to respond to FLMP (11). As demonstrated by other authors (12), FMLP induces chemotaxis only when EOS are primed by different cytokines, such as GM-CSF and IL-3. Thus, since lymphocytes of allergic patients, even spontaneously, secrete IL-3 and GM-CSF (21), it is obvious that EOS from allergic patients are primed to respond to FMLP. Indeed, as in our present study, Warringa et al. (12) have demonstrated that EOS from allergic patients, in contrast to those of normal subjects, are able to respond to FMLP. Moreover, our results demonstrate that, while before and after season the EOS chemotactic response to C5a of allergic patients is comparable to that seen in controls, a significant reduction occurs during the pollen season, particularly at the peak pollen count.

This difference seems to conflict with the findings for FMLP, but it may reflect the complex regulation of chemotactic capacity of allergic patients. It has been hypothesized that histamine may modulate these cellular functions (14). Indeed, a low histamine concentration can enhance mitogenesis and chemotaxis, while high doses reduce the proliferative effect and the chemotactic capacity (15, 22, 23). This dual histamine effect on chemotaxis, confirmed in the present study, may be due to a modulation of different receptors (23). It has been observed that, while histamine at higher concentrations induces, through H_2 -receptor binding, an impairment of cellular functions, enhancing the intracellular levels of cAMP, at lower concentrations, histamine, by the modulating H_1 receptor, increases intracellular cGMP levels, improving

cellular microtubule assembly capacity, cytoskeleton integrity, and polarization (22).

Thus, our present results suggest that the C5a-induced chemotactic activity during the *Parietaria*-pollen season may be correlated with the *in vivo* presence of high histamine levels. This hypothesis has been confirmed by our *in vitro* study (Table 2). On the other hand, the increase of serum histamine, an index of mast-cell degranulation and bronchial challenge, has been reported to occur *in vivo* during the season (13, 24, 25). Moreover, the hypothesis is further supported by the modulating effects obtained with cimetidine, an inhibitor of H₂ receptors, both *in vivo* and *in vitro*. Finally, the *in vitro* suppression of C5a-induced chemotaxis observed with diphenhydramine in the presence of low histamine concentrations seems to be in accordance with the hypothesis that the reduction of serum histamine, after and before the season, might enhance EOS chemotaxis.

In conclusion, our results indicate that histamine at different doses may play an important role in the modulation of EOS chemotactic capacity and that H₁- and H₂-receptor antagonists may reverse these effects.

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