

Nasal response to a single antigen challenge in patients with allergic rhinitis — inflammatory cell recruitment persists up to 48 hours

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Summary

Background Allergen challenge in some patients with respiratory allergy is followed by an early and a late reaction.

Objective To evaluate the duration of mediator release and inflammatory cell recruitment during the late antigen-induced nasal response.

Methods Eight patients with seasonal allergic rhinitis due to grass pollen underwent local challenge with the relevant allergen, a non-relevant allergen (*Parietaria judaica*), and nebulized saline solution. Nasal lavages were performed at baseline and 6, 24, 48, 72 h after challenge. Eosinophil cationic protein (ECP), leukotriene C₄ (LTC₄), leukotriene B₄ (LTB₄) myeloperoxidase (MPO) and prostaglandin D₂ (PGD₂) levels were radioimmunoassayed and histamine concentration was measured by an automated fluorometric method.

Results Nasal challenge with the relevant antigen induced a response 6 h after stimulation, which subsided within 24 h. Eosinophilia, observed in the nasal lavages collected from 6 to 24 h after this challenge, was accompanied by ECP release. Neutrophilia were found in the nasal lavages collected from 6 to 24 h after challenge. The increase in neutrophil number correlated with MPO levels and LTB₄ concentrations, but not with the intensity of nasal obstruction. Antigen challenge also induced significant recruitment of mononuclear cells 48 h after provocation. The challenge significantly raised histamine, but not PGD₂, levels in the nasal lavages collected 6 h after provocation. A trend towards an increase in LTC₄ levels in the nasal lavages collected 6 h after specific antigen challenge was also found. Nasal challenge with a non-relevant allergen or with saline solution did not cause either inflammatory cell recruitment or mediator release.

Conclusion Nasal challenge with the relevant antigen can induce a late response characterized by local accumulation of eosinophils, neutrophils and mononuclear cells persisting for 48 h and accompanied by release of ECP, MPO, LTB₄ and histamine. These results indicate that a single antigen challenge in patients with allergic rhinitis causes prolonged inflammatory alterations which may contribute to the development of airway hyperreactivity.

Keywords: allergic rhinitis, late reaction, mediator release

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Introduction

Challenge with the relevant antigen in patients suffering from respiratory allergy provokes an immediate response characterized by rhinorrhoea, itching, sneezing and

obstruction in the nose, by bronchospasm in the lower airways and a weal and flare reaction in the skin. Mast cells play an important role in the immediate reaction in the nose, since they rapidly release inflammatory mediators such as histamine, LTC₄ and PGD₂, after membrane IgE cross-linking by antigen [1,2]. These mediators can increase vascular permeability, and cause vasodilation, hyper-secretion and nasal obstruction, effects which are responsible for the allergic symptoms. These mediators have different effects when insufflated in the nose. Nasal challenge with histamine causes itching, sneezing, rhinorrhoea and nasal obstruction, while LTC₄ and PGD₂ cause mainly nasal obstruction [2,3].

The response to antigen stimulation is dual in 40–50% of the patients, an early reaction being followed by a delayed symptomatic response or late-phase reaction, which develops between 3 and 6 h, peaks at 6–8 h and subsides 12–24 h after allergen challenge [4]. The late reaction in the skin was shown to be IgE-dependent, meaning it requires the antigen-induced IgE-dependent activation of mast cells [5,6]. Probably, the same mechanism underlies the late antigen-induced reaction in the nose, although some data suggest other mechanisms can be involved [7]. Recruitment of inflammatory cells and inflammatory mediator release both contribute to appearance and maintenance of the late response [8–10]. The eosinophils play an important role in the late reaction. De Monchy *et al.* [11] observed a close relationship between eosinophil recruitment and the late reaction in the lower airways. The association between eosinophils at the site of antigen challenge and appearance of the late reaction has also been demonstrated in the skin [12]. Eosinophils can release toxic mediators, which can cause epithelial damage [13] and may contribute to the late antigen-induced reaction and target organ hyperreactivity [14].

The aim of this study was to investigate the duration of nasal inflammation after challenge with the relevant antigen in patients with allergic rhinitis. We investigated inflammatory cell recruitment and measured some inflammatory mediators, such as histamine, LTC₄, PGD₂, ECP and MPO.

Subjects and methods

Subjects

Eight patients with seasonal allergic rhinitis (six men and two women; 17–41 years of age) due to grass pollen were included in this study. Allergic rhinitis was diagnosed on the basis of clinical history, skin prick tests, a positive nasal antigen challenge and detection of serum-specific IgE antibodies to grass pollen by the radioallergosorbent test (Cap system, Pharmacia-Upjohn, Uppsala, Sweden). All patients had a documented late response to nasal antigen

provocation. Our study was carried out in winter, when the patients were free of symptoms. The patients had not received immunotherapy in the past and were not taking any systemic or local medication. The study was approved by the institutional review board and an informed consent was obtained from each person before the study.

Nasal challenge and nasal lavage procedure

The patients underwent nasal challenge with the relevant antigen (2000 biological units of lyophilized purified grass pollen extract diluted in saline solution, prepared by Lofarma, Milan, Italy). To evaluate whether changes in nasal cytology and mediator release were non-specific, the patients also underwent nasal challenge with a non-relevant antigen (2000 biological units of *Parietaria judaica* extract diluted in saline solution, prepared by Lofarma, Milan, Italy) and saline solution (sterile 0.9% w/v NaCl solution). All challenges were performed in a double-blind fashion. The stimuli were insufflated in both nostrils by a nasal spray pump delivering $100 \pm 10 \mu\text{L}$ for each puff. Nasal lavages were performed at baseline and at different times after challenge (6, 24, 48 and 72 h) to evaluate mediator release and changes in nasal cell populations.

Nasal lavage fluids were collected according to a technique previously described [2,15]. Briefly, 5 mL of saline solution (0.9% w/v NaCl) at 37 °C was instilled into each nostril with a syringe, while the patient extended his neck, and abstained from breathing or swallowing. After 10 s the subject expelled the mixture of mucus and saline, which was collected in conical polypropylene tubes (Falcon, Becton Dickinson Labware, Oxnard, CA, USA) and kept on ice until the end of the experiment. Recovery of nasal lavage fluid ranged between 60 and 80% of the starting volume (10 mL for each washing). Soon after collection, nasal lavage fluids were shaken to disperse mucus and were centrifuged at 4 °C for 10 min at 300 *g* to eliminate the mucus layer; the supernatant of each sample was aspirated and then centrifuged at 4 °C for 15 min at 2000 *g*, divided into aliquots, and stored at –80 °C. The cell pellet was resuspended in 1 mL of RPMI with HEPES (Sigma Chemicals, St Louis, Mo, USA) and 0.1% human serum albumin (w/v) (Sigma Chemicals).

Total cell counts were done in duplicate in a Fuchs-Rosenthal chamber (Walter Schrenk, Hofheim am Taunus, Germany) after staining with Turk liquid (50 μL sample and 450 μL Turk liquid prepared by Farmacia Ospedale Policlinico, Milan, Italy). Eosinophils were counted by Randolph staining (solutions provided by Farmacia Ospedale Policlinico) [16]. Two cytopsin slides were prepared for each sample (100 μL with $2\text{--}4 \times 10^4$ cells for each slide; centrifugation at 22.4 *g* for 10 min in a Shandon cytocentrifuge, Shandon Southern Ltd, Runcorn, UK). Cytocentrifuge

slides were stained by May-Grunwald Giemsa (staining solutions provided by Farmacia Ospedale Policlinico) and examined with a light microscope under oil immersion. At least 200–300 cells were examined in each slide. The cells were classified as epithelial cells, neutrophils, eosinophils and mononuclear cells (the latter category included lymphocytes, monocytes, and mononuclear cells of unclear origin). The reader was blinded as to whether the subject had undergone nasal challenge with the relevant antigen, the non-relevant antigen or saline solution.

Record of symptoms

Patients were asked to record the severity of rhinorrhoea, itching, sneezing and nasal obstruction after nasal insufflation of the relevant antigen, the non-relevant antigen or saline solution. These symptoms were rated on an arbitrary scale from 0 to 4 (none = 0, mild = 1, moderate = 2, intense = 3, very intense = 4). Sneezes were counted and classified as 0 = no sneezes, 1 = 1–5 sneezes, 2 = 6–10 sneezes, 3 = 11–15 sneezes, 4 $\geq$ 15 sneezes. Nasal obstruction, which was the main symptom complained during the late reaction, was reported separately; the scores for the other symptoms were combined.

Mediator assays

Histamine

Aliquots of the supernatants from nasal lavage fluids were mixed with one volume of 4% v/v perchloric acid, centrifuged at 4 °C for 15 min at 2000 *g*, and assayed in duplicate for histamine by an automated fluorometric technique [17]. The sensitivity of this method is 1 ng/mL.

LTC₄

Aliquots of the supernatants for LTC₄ assay were aspirated, decanted and extracted following the method of Anderson *et al.* [18]. The lavage supernatants were mixed with four volumes of absolute ethanol (Carlo Erba-Pharmacia-Upjohn, Milan, Italy) and left at 2–8 °C for 30 min. The protein-rich precipitate was removed by centrifugation at 3000 *g* for 30 min at 4 °C; the ethanol supernatant was collected, decanted and removed by rotary evaporation under vacuum. The samples were stored at –20 °C, then redissolved in distilled water for radioimmunoassay.

Immunoreactive LTC₄ was radioimmunoassayed, using commercially available kits (Amersham, Little Chalfont, UK), according to Hammond *et al.* [19]. The anti-LTC₄ antibody employed in the assay cross-reacts with LTD₄ (64%) and LTE₄ (64%). The antibody-antigen complexes were separated from the free antigen by adsorption of the free tracer onto activated charcoal. After centrifugation, the supernatant containing the antigen-antibody complexes

was decanted into a polyethylene vial, and 10 mL of a liquid scintillation cocktail (Insta-gel, Packard Instrument Co., Meriden, CT, USA) was added. The radioactivity was counted in a β -counter (Tri-Carb 4000, Packard Instrument Co.).

The sensitivity of the assay is 0.125 ng/mL and intra-assay and interassay variations did not exceed 10%. Every sample was assayed in duplicate and the results were expressed as ng/mL lavage fluid. The results of radioimmunological determination of LTC₄ in nasal washing were previously validated by high performance liquid chromatography, according to Sautebin *et al.* [20], already described elsewhere [2].

PGD₂

Aliquots of the nasal washings were evaporated to dryness under a stream of nitrogen and PGD₂ was extracted according to Powell [21]. PGD₂ was determined by a competitive radioimmunological method, using a commercially available kit (Amersham).

The sensitivity of the assay is 0.030 ng/mL and intra-assay and interassay variations did not exceed 10%. Every sample was assayed in duplicate and the results were expressed as ng/mL lavage fluid.

LTB₄

Aliquots of the supernatants for LT assay were aspirated, decanted and extracted following the method of Anderson *et al.* [18]. The lavage supernatants were mixed with four volumes of absolute ethanol (Carlo Erba-Pharmacia-Upjohn) and left at 2–8 °C for 30 min. The protein-rich precipitate was removed by centrifugation at 3000 *g* for 30 min at 4 °C; the ethanol supernatant was collected, decanted, then removed by rotary evaporation under vacuum. The samples were stored at –20 °C and redissolved in distilled water for radioimmunoassay. Immunoreactive LTB₄ was radioimmunoassayed, using commercially available kits (Amersham), according to Simmons *et al.* [22]. The antibody-antigen complexes were separated from the free antigen by adsorption of the free tracer onto activated charcoal. After centrifugation, the supernatant containing the antigen-antibody complexes was decanted into a polyethylene vial, and 10 mL of a liquid scintillation cocktail (Insta-gel, Packard Instrument Co.) was added. The radioactivity was counted in a β -counter (Tri-Carb 4000, Packard Instrument Co.). The LTB₄ rabbit antiserum does not cross-react with peptide-leucotrienes, but cross-reacts as much as 3.9% with diastereoisomers of LTB₄. The sensitivity of the assay is 16 pg/mL and intra-assay and interassay variations did not exceed 10%. Every sample was assayed in duplicate and the results were expressed as ng/mL lavage fluid.

ECP

The concentration of ECP was measured by

radioimmunoassay, using a commercially available kit (Pharmacia-Upjohn), which followed the method developed by Venge *et al.* [23]. The sensitivity of the assay is 2 ng/mL and cross-reactivity of the anti-ECP rabbit antiserum is less than 0.06% with eosinophil peroxidase.

MPO

The concentration of MPO was measured by radioimmunoassay, using a commercially available kit (Pharmacia-Upjohn). The sensitivity of the assay is 8 ng/mL and cross-reactivity of the anti-MPO antiserum is less than 0.1% with lactoferrin, lysozyme, ECP and eosinophil peroxidase.

Statistical analysis

Results were expressed as median and range. Friedman's non-parametric repeated measures test and Dunn's multiple comparisons test were used to analyse the changes in inflammatory cell numbers and mediator levels in nasal lavages (prechallenge values were compared with values obtained at different times after challenge). When the results fell below the limit of detection of the assay, statistical significance was calculated using the lowest detectable value for that assay. Spearman rank correlation analysis was used to analyse the correlation between the parameters examined. *P*-values less than 0.05 were considered significant.

Results

Nasal symptoms

Nasal challenge with the relevant antigen provoked an early nasal reaction in all patients (Fig. 1). Rhinorrhoea, itching and sneezing reached the maximum score 5–10 min after challenge, persisted until 30 min after stimulation and subsided gradually within 60 min. Maximum nasal obstruction was reported 10–20 min after antigen challenge and subsided after 60 min.

By 6 h after challenge with the relevant allergen, all patients complained of a late reaction, consisting mainly of nasal obstruction. The other symptoms during the late nasal reaction were mild. By 24 h all symptoms had disappeared.

Nasal provocation with the non-relevant antigen or with saline solution did not provoke any nasal symptom.

Cellular counts in nasal lavage fluids

Nasal stimulation with the relevant antigen determined an increase in total cell counts in the nasal lavages collected from 6 to 24 h after provocation (baseline: 232.5×10^3 [90×10^3 – 750×10^3]; 6 h: 725×10^3 [400×10^3 – 1655×10^3],

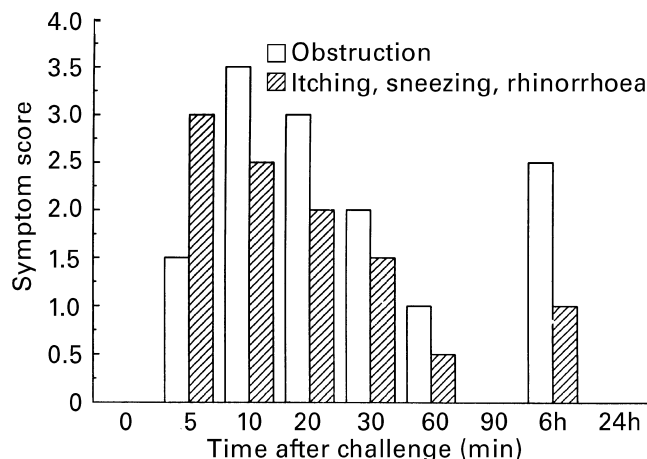


Fig. 1. Time course of symptom scores in patients with allergic rhinitis after challenge with the relevant antigen. All symptoms subsided within 60 min, but 6 h after challenge a late reaction, consisting mainly of nasal obstruction, was observed. The results are expressed as medians.

$P < 0.001$; 24 h: 682.5×10^3 [200×10^3 – 1460×10^3] $P < 0.01$; 48 h: 635×10^3 [220×10^3 – 1000×10^3]; 72 h: 325×10^3 [125×10^3 – 520×10^3]. No significant changes in total cell counts were induced by stimulation with a non-relevant antigen or with saline solution. Differential cell counts in prechallenge and post-challenge nasal lavages are shown in Table 1.

Challenge with the relevant antigen induced significant nasal neutrophilia, which appeared 6 h ($P < 0.001$) after provocation and lasted until 24 h ($P < 0.05$). No significant increase in neutrophil counts was observed after stimulation with the non-relevant antigen or with saline solution. The correlation between neutrophil counts and the scores for nasal obstruction was not significant.

Nasal antigen challenge provoked significant eosinophilia in the nasal lavages collected from 6 ($P < 0.01$) to 24 h ($P < 0.01$). Eosinophil number was not affected by provocation with the non-relevant antigen or saline solution.

Challenge with the relevant antigen induced significant recruitment of mononuclear cells at 48 h after specific antigen stimulation ($P < 0.05$).

Mediator levels in nasal lavage fluids

Histamine

Median histamine concentrations in prechallenge lavages were 28.5 ng/mL (range 1–48) before stimulation with the relevant antigen, 21.5 ng/mL (range 3–41) before saline insufflation and 32.5 ng/mL (range 4–50) before provocation with the non-relevant allergen. Histamine was significantly increased in the nasal washings collected 6 h after stimulation with the relevant antigen ($P < 0.01$). Then they

Table 1. Cell counts (epithelial cells, neutrophils, eosinophils and mononuclear cells) in nasal lavage fluids obtained from allergic rhinitic patients before stimulation and at different times (6, 24, 48 and 72 h) after stimulation with relevant antigen, non-relevant antigen and saline solution. The results are expressed as median (range).

	Epithelial cells	Neutrophils	Eosinophils	Mononuclear cells
<i>Relevant antigen</i>				
Baseline	184.4 (54.9–304.9)	65.5 (19–440)	0.1 (0.1–7.5)	5.5 (1–10)
6 h	241.25 (147–400)	447.1*** (204–1340)	52.5** (15.2–100)	7.75 (0–20)
24 h	175 (69–592)	351.25* (35–870)	72.5** (2.5–253)	10 (0–30)
48 h	199.5 (68.2–416)	118 (39.6–810)	47.8 (5–142.5)	39* (10–97.5)
72 h	185.7 (60–305)	53.75 (39–200)	17.5 (0.1–62.5)	29.25 (10–56)
<i>Non-relevant antigen</i>				
Baseline	215 (94–300)	130 (20–200)	2 (0–6)	2.5 (0–9)
6 h	230 (300–310)	140 (830–210)	3 (0–10)	5.5 (0–9)
24 h	230 (100–300)	135 (25–190)	3.5 (0–10)	5 (0–9)
48 h	220 (100–300)	135 (20–200)	3.5 (0–10)	5 (0–9)
72 h	225 (90–300)	135 (20–200)	3.5 (0–10)	5 (0–9)
<i>Saline solution</i>				
Baseline	166 (100–232)	105 (30–150)	2 (0–3)	3 (0–6)
6 h	180 (100–330)	100 (37–161)	2 (0–4)	4.5(0–7)
24 h	165 (310–320)	105 (47–174)	2 (0–3)	2 (0–8)
48 h	170 (100–370)	95 (27–143)	1 (0–6)	1 (0–6)
72 h	180 (120–320)	100 (32–153)	1 (0–4)	2.5 (0–6)

* $P < 0.05$ vs baseline. ** $P < 0.01$ vs baseline. *** $P < 0.001$ vs baseline.

dropped back to baseline. Histamine concentrations were unchanged after challenge with the non-relevant antigen or saline solution (Fig. 2).

LTC₄

Median LTC₄ levels in the nasal lavages collected at baseline were 0.58 ng/mL (range 0.17–1.2) before stimulation with the relevant antigen, 0.43 ng/mL (range

0.13–1.14) before saline insufflation and 0.52 ng/mL (range 0.12–1.24) before provocation with the non-relevant allergen. Nasal challenge with the relevant antigen did not cause any significant release of LTC₄ in the nasal washings collected at indicated times, although a trend towards increased levels was found 6 h after challenge. There was no increase in LTC₄ in the nasal lavages collected after challenge with the non-relevant antigen or saline solution (Fig. 3).

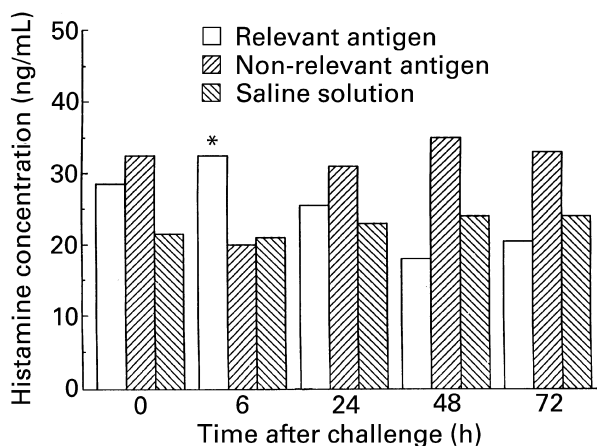


Fig. 2. Time course of histamine concentration in nasal lavage fluids collected after stimulation with a relevant antigen, a non-relevant antigen, or saline solution. The results are expressed as medians. * $P < 0.01$ vs baseline

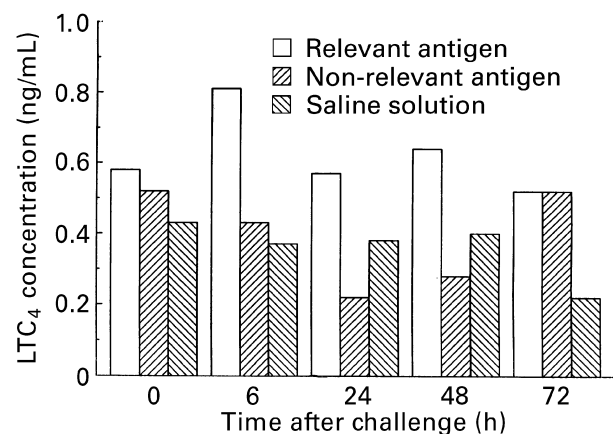


Fig. 3. Time course of LTC₄ concentration in nasal lavage fluids collected after stimulation with a relevant antigen, a non-relevant antigen, or saline solution. The results are expressed as medians.

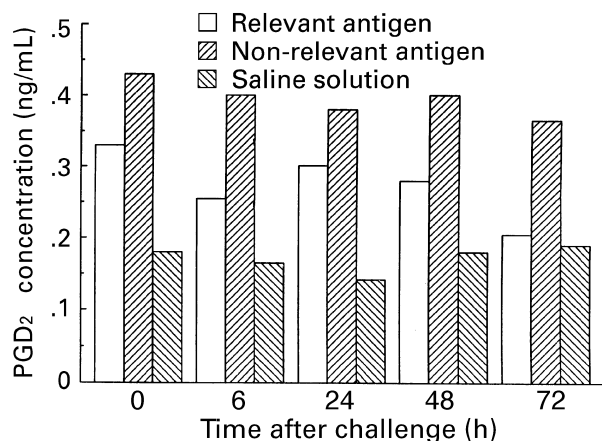


Fig. 4. Time course of PGD₂ concentration in nasal lavage fluids collected after stimulation with a relevant antigen, a non-relevant antigen, or saline solution. The results are expressed as medians.

PGD₂

Median PGD₂ concentrations were 0.33 ng/mL (range 0.1–0.5) before stimulation with the relevant antigen, 0.18 ng/mL (range 0.03–0.84) before saline insufflation and 0.43 ng/mL (range 0.03–0.72) before provocation with the non-relevant allergen. Nasal challenge with the relevant antigen did not cause any significant release of PGD₂ in the nasal washings collected at indicated times. The time course of PGD₂ concentrations was similar after stimulation with the relevant antigen, with the non-relevant antigen and saline solution (Fig. 4).

LTB₄

LTB₄ levels in the nasal lavages collected under basal conditions were 0.42 ng/mL (median, range 0.21–0.75) before stimulation with the relevant antigen, 0.35 ng/mL (median, range 0.05–1) before saline insufflation and 0.38 ng/mL (median, range 0.21–0.71) before provocation with the non-relevant allergen. A significant increase in LTB₄ concentration was observed in the nasal lavage fluids collected from 6 ($P < 0.001$) to 24 h ($P < 0.05$) after provocation with the relevant antigen. By 72 h, LTB₄ levels were similar to the basal values. A significant correlation was observed between neutrophil counts and LTB₄ levels in the nasal lavages collected from 6 to 48 h after challenge with the relevant antigen (6 h: $r = 0.73$, $P < 0.05$; 24 h: $r = 0.77$, $P < 0.05$; 48 h: $r = 0.99$, $P < 0.0001$).

LTB₄ concentrations did not change after challenge with the non-relevant antigen or with saline solution (Fig. 5).

MPO

Median MPO concentrations in prechallenge lavages were 88.05 ng/mL (range 16.4–239.5) before stimulation with the relevant antigen, 91.55 ng/mL (range 8–326.3) before saline insufflation and 125.7 ng/mL (range 24.2–310.8) before

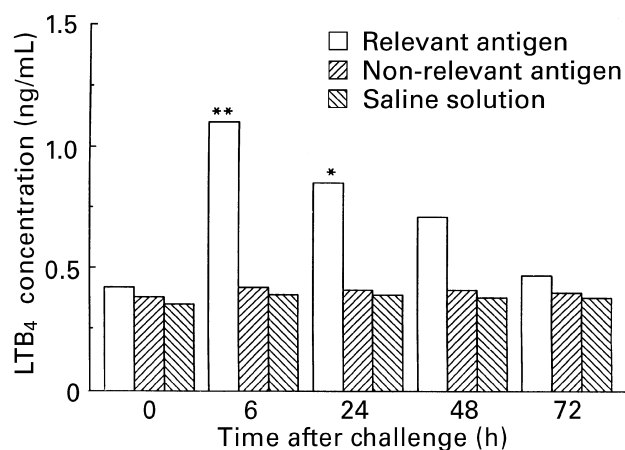


Fig. 5. Time course of LTB₄ concentration in nasal lavage fluids collected after stimulation with a relevant antigen, a non-relevant antigen, or saline solution. The results are expressed as medians. * $P < 0.05$ vs baseline ** $P < 0.001$ vs baseline

provocation with the non-relevant allergen. A significant increase in MPO from basal value was found 6 h ($P < 0.05$) after challenge with the relevant antigen. A significant correlation was observed between neutrophil counts and MPO concentrations in the nasal lavages collected 6 ($r = 0.73$, $P < 0.05$), 24 ($r = 0.83$, $P < 0.01$) and 48 h ($r = 0.92$, $P < 0.005$) after challenge with the relevant antigen.

MPO concentration did not increase in the nasal lavages collected after challenge with the non-relevant antigen or saline solution (Fig. 6).

ECP

ECP concentration in prechallenge lavages was 3.25 ng/mL (median, range 2–14.6) before stimulation with the relevant

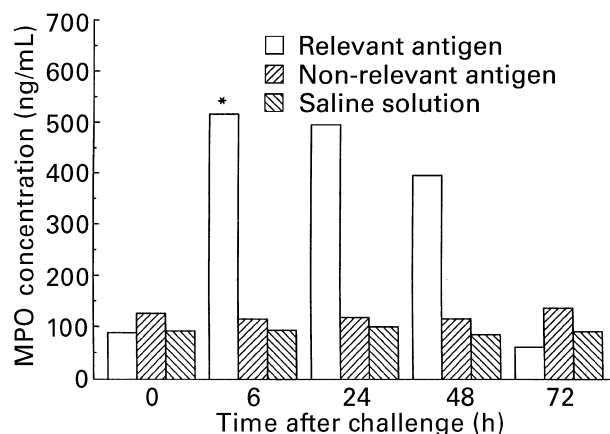


Fig. 6. Time course of MPO concentration in nasal lavage fluids collected after stimulation with a relevant antigen, a non-relevant antigen, or saline solution. The results are expressed as medians. * $P < 0.05$ vs baseline

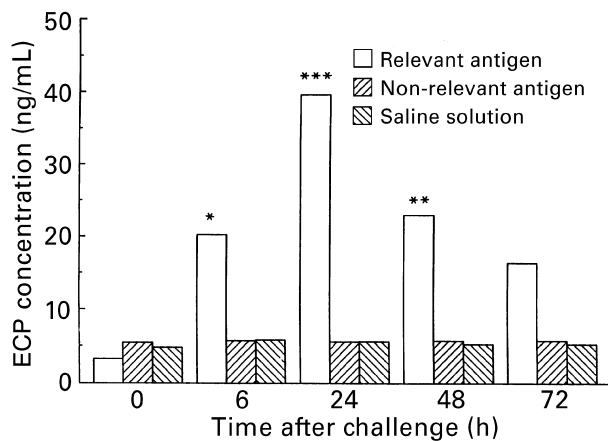


Fig. 7. Time course of ECP concentration in nasal lavage fluids collected after stimulation with a relevant antigen, a non-relevant antigen, or saline solution. The results are expressed as medians. * $P < 0.05$ vs baseline, ** $P < 0.01$ vs baseline, *** $P < 0.001$ vs baseline.

antigen, 4.8 ng/mL (median, range 2.3–9.8) before saline insufflation and 5.4 ng/mL (median, range 2–10.5) before provocation with the non-relevant allergen. Nasal antigen challenge significantly raised ECP levels in the nasal lavages 6 ($P < 0.05$), 24 ($P < 0.001$) and 48 h ($P < 0.01$) after stimulation. A significant correlation was observed between eosinophil count and ECP levels from baseline up to 48 h after specific antigen challenge (baseline: $r = 0.78$, $P < 0.02$; 6 h: $r = 1$, $P < 0.0001$; 24 h: $r = 0.92$, $P < 0.001$; 48 h: $r = 0.88$, $P < 0.002$).

ECP concentration did not change after challenge with the non-relevant antigen or saline solution (Fig. 7).

Discussion

In some patients with respiratory allergy, allergen challenge is followed by an early reaction and a late reaction, occurring about 6 h after allergen exposure. The late reaction closely recalls the alterations seen in patients with chronic allergic diseases, and is currently believed to be the underlying mechanism of subacute/chronic allergic inflammation. This study investigated the mechanisms leading to the late antigen-induced reaction in patients with allergic rhinitis. Prolonging the observation of the patients up to 72 h after challenge, we found that a single allergen exposure in these patients with allergic rhinitis, known to be dual responders (i.e. presenting an early and a late reaction), was followed by recruitment of inflammatory cells, mostly eosinophils and neutrophils, and release of inflammatory mediators persisting for 48 h.

The role of eosinophils and eosinophil-derived mediators in the late antigen-induced reaction is recognized and this is in fact considered one of the most important cells in the

development of allergic diseases [14]. Our findings indicate that eosinophils are attracted into the nasal airways and can be found in increased numbers in the nasal lavages collected from 6 to 24 h after antigen challenge. ECP concentrations were increased from 6 to 48 h after challenge and were positively correlated with eosinophil numbers. However, nasal symptoms peaked 6 h after challenge, and disappeared within 24 h. By 24 h, in spite of the presence of ECP and eosinophils in nasal lavages, the patients were symptom-free. Therefore, ECP seems not to play an important role in nasal obstruction, whereas it can damage nasal mucosa and contribute to the development of nasal hyperreactivity [13,14]. It is interesting to note that ECP, but not LTC₄, was found in increased amounts at 6, 24 and 48 h after antigen challenge. Since infiltrating eosinophils are presumably activated, our failure to find increased amounts of LTC₄ could be due to (a) a more rapid degradation of LTC₄ than ECP; (b) rapid binding of LTC₄ to specific receptors, with low levels of free LTC₄ in nasal lavages; or (c) differences in release kinetics between LTC₄, which is a newly generated mediator, and ECP, which is a preformed mediator.

Nasal challenge with the relevant antigen in allergic patients was followed by significant accumulation of neutrophils, which were activated, since they released MPO and the newly synthesized lipid mediator LTB₄. Neutrophils seem therefore to participate in allergic inflammation, although their number was not notably correlated with nasal obstruction. Non-specific antigen challenge caused slight, but not significant, neutrophilia, which was the expression of a non-specific reaction.

The role of neutrophils in allergic diseases is much debated. Some authors suggest that the neutrophil is involved in allergic reactions [24,25], whereas others believe it is attracted to the site of allergic inflammation as the result of non-specific stimulation [9]. Probably, both theories are true, meaning that the neutrophil is recruited at the site of non-specific stimulation, but it can also have a role in the development of allergic reactions, since it is activated and releases inflammatory mediators after antigen challenge in allergic patients [26,27].

A significant increase in histamine, but not PGD₂, was observed in the nasal lavages collected 6 h after stimulation with the relevant allergen. A trend towards increased LTC₄ levels was found 6 h after challenge. These findings agree with Naclerio *et al.* [8] who found an increase in histamine, but not PGD₂, in nasal washings collected during the late reactions. This pattern of mediator release suggests that basophils, and not mast cells, are activated during the late reactions [28]. Basophils can, in fact, release histamine and LTC₄ but not PGD₂, which is produced by mast cells.

Recruitment of mononuclear cells was seen only in the nasal lavages collected 48 h after antigen provocation. This

finding is in line with Varney *et al.* [29] who observed an increase in CD4⁺CD25⁺ T helper cells in biopsies taken 24 h after nasal stimulation with the relevant antigen. Also in ocular allergy, the accumulation of lymphocytes has been observed during the late phase reaction [30]. Although the participation of mononuclear cells is numerically less important and the recruitment is slower than that of eosinophils, these cells do appear to have some role in the development and perpetuation of allergic inflammation. Activated T cells have been found in blood, bronchial mucosal biopsies and bronchoalveolar lavage fluids from allergic asthmatics [31–33]. These cells synthesize and release cytokines and chemotactic factors which in turn attract and activate other inflammatory cells and induce the expression of adhesion molecules. Cytokines, such as interleukin-3, -5 and granulocyte macrophage-colony stimulating factor (GM-CSF) can influence eosinophil and basophil differentiation, attraction and activation, and might contribute to the recruitment of these cells which is characteristically observed at the site of allergen challenge during late reactions [34–36]. Another important chemoattractant and activator for eosinophils and neutrophils is platelet activating factor (PAF), a lipid mediator synthesized by several inflammatory cells, when activated through immunological mechanisms. When insufflated in the nose, PAF can induce local neutrophilia and, to a lesser extent, eosinophilia [37].

In conclusion, our study found that the late antigen-induced nasal reaction was associated with local accumulation of eosinophils, neutrophils and mononuclear cells persisting up to 48 h. The raised amounts of ECP, MPO and LTB₄ indicated that either eosinophils or neutrophils are activated and release their inflammatory mediators. A single antigen challenge in patients with allergic rhinitis can induce lasting inflammatory alterations in the nose, which may exert a priming effect on nasal allergic reactions. Iliopoulos *et al.* [38] showed that a single antigen challenge can induce an enhancement of clinical symptoms and an increased mediator release on rechallenge. The prolonged changes in inflammatory cells and mediators that we have found may help us understand the mechanisms underlying allergic inflammation and airway hyperreactivity.

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