

ORIGINAL ARTICLE

Myeloperoxidase Release After Allergen-Specific Conjunctival Challenge

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ABSTRACT

Background: Allergen-specific conjunctival challenge is a fruitful and complete tool in evaluating pathophysiological phenomena of allergic inflammation. After challenge, a significant neutrophil infiltrate occurred in allergic subjects. The primary (azurophilic) granules of neutrophils contain a variety of enzymes that might potentiate inflammation, such as myeloperoxidase (MPO). It is not known whether allergen-specific conjunctival challenge (ASCC) is able to elicit MPO release. We also investigated the possible role of immunotherapy (IT) in the release of MPO. **Method:** The groups studied included *Dactylis glomerata*-sensitive adult atopic patients suffering from seasonal allergic rhinoconjunctivitis, and healthy adult nonatopic volunteer controls. One group of allergic patients received no specific hyposensitization (not-IT allergic group). A second group of allergic patients had been immunotherapy-treated with *Dactylis glomerata* extract for the preceding three years and continued to receive a maintenance dose within the highest potency of the extract (IT-allergic group). ASCC with *Dactylis glomerata* was performed outside the pollen season in all subjects. Myeloperoxidase was assayed by MPO-enzyme immunoassay method. **Results:** Thirty minutes after challenge, myeloperoxidase levels in the non-immunotherapy allergic patients were significantly higher compared than in the healthy group ($p < 0.001$). The levels of myeloperoxidase released in the immunotherapy allergic group were significantly lower than those in the nonimmunotherapy allergic group ($p < 0.001$) and higher than those in non-allergic subjects ($p < 0.001$). **Conclusion:** These results indicate that after ASCC

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there is a release of MPO. Our study suggests that immunotherapy actively modifies the release of MPO after ASCC.

Key Words: Allergy; Rhinoconjunctivitis; Neutrophils; Mechanism; IgE-dependent; Allergen; Myeloperoxidase; Release; Ocular; Conjunctiva; Allergen challenge.

INTRODUCTION

The eye model is different from the bronchial one, since the conjunctiva has no muscles. Nevertheless, the development of clinical and inflammatory changes following allergen-specific challenge may be superimposable, as inflammatory cell infiltration and mediator release occur similarly in the target organ of allergic reaction, whether skin, eye, nose, or bronchi (1). The eye model creates a unique opportunity to monitor clinical and inflammatory changes following allergen challenge in the same patients at various times. Therefore, allergen-specific conjunctival challenge (ASCC) provides a useful tool to confirm the diagnosis of sensitization to a specific allergen, to evaluate the grade of specific sensitization, and to study the pathophysiology of allergic reactions considering signs and symptoms, cells and mediators of the inflammatory response (2,3). There is increasing evidence of neutrophil participation in asthma and the allergic process. Thirty minutes after ASCC, a statistically significant inflammatory neutrophil infiltrate occurred in allergic subjects when compared with baseline conditions and with the other study group. Neither clinical nor cytological reactions occurred in the contralateral eye challenged with diluent (4,5). Neutrophils possess a number of cytotoxic enzymes and associated pathways which can be utilized for cell-killing. One such cytotoxic system is provided by myeloperoxidase (MPO), a hemoprotein located within azurophilic granules. It is not known whether ASCC is able to elicit MPO release. In this study, we attempted to evaluate the release of MPO after ASCC in allergic patients. We also investigated the possible role of immunotherapy (IT) in the release of MPO.

PATIENTS AND METHODS

Study Subjects

The groups studied included *Dactylis glomerata*-sensitive adult atopic patients suffering from seasonal allergic rhinoconjunctivitis, and healthy adult nonatopic volunteer controls. One group of allergic patients

(n=10) had positive skin prick test (Bial-Aristegui, Bilbao, Spain) and specific IgE (HYTEC 288, Hycor Biomedical Inc., Irvine, CA, USA) to *Dactylis glomerata*. The subjects received no immunotherapy treatment (not-IT allergic group). A second group of allergic patients (n=10)—also *Dactylis glomerata*-sensitive—had been immunotherapy-treated with *Dactylis glomerata* extract for the preceding three years and continued to receive a maintenance dose within the highest potency of the extract (IT allergic group). The healthy controls (n=10) had no history of allergy or bronchial symptoms, and had negative skin prick test (Bial-Aristegui) and normal specific IgE (HYTEC 288) to a battery of inhalant allergens (house-dust mites, pollens, molds, and animal danders). On admission, all subjects had no ocular or nasal allergic or non-allergic diseases, and they did not wear contact lenses. The study was performed outside the pollen season, when *Dactylis glomerata*-sensitized patients were symptom-free. No topical or systemic drugs were allowed for 1 month before and during the study period. The Hospital Ethics Committee approved the study, and each subject gave informed consent.

ASCC

The allergen—commercially available antigen freeze-dried standardized extracts from orchard grass (*Dactylis glomerata*)—was purchased from Bial-Aristegui. Extracts were labeled in biological units (BU), using a method derived from the proposals of the “Nordic Council of Medicines Guidelines on Allergen Standardization (6). The freeze-dried extracts were stored at +4°C. The same batch of allergen extract was used throughout the study. Each test day, a new vial was diluted in sterile isotonic saline albumin diluent, which was prepared without preservatives.

On entering the clinic, all subjects were examined to establish that no visible ocular symptoms were present at the time of challenge. ASCC was performed as previously described (2). Briefly, 10 µL of diluted allergen (Bial-Aristegui) were instilled into the lower conjunctival sac in the right eye, and saline albumin diluent, used to dilute the allergen, was administered into the contralateral eye to exclude possible nonspecific

reactivity. The initial allergen concentration was the same as in the skin end-point test, according to the ASCC protocol (2). Six different dilutions were used during ASCC: 1, 10, 30, 60, 90, and 100 UB/mL. Every 15 min, the doses of allergen was increased until an early clinical reaction was elicited [total symptom score ≥ 7 was considered positive as previously described (2)]. Clinical conjunctival evaluation was performed at baseline and 30 min after challenge. Conjunctival hyperemia, lacrimation, itching-burning, and swelling of eyelids were scored according to an arbitrary 4-point rating scale, from 0 to 3 (0=absent, 1=mild, 2=moderate, and 3=severe); this score was proposed by Abelson et al. [Ref. (7); Table 1]. Signs were evaluated in blinded fashion by two clinicians, symptoms were spontaneously reported by the subjects, and a total symptom score was calculated as the sum of individual symptom scores (2). After clinical evaluation of ocular signs and symptoms, tear samples were collected using a capillary micropipette, centrifuged, and stored at -20°C until assay was performed (8). Healthy subjects underwent ASCC with use of the highest allergen concentration (100 UB/mL).

Table 1. Scoring system to measure the signs and symptoms of allergic conjunctivitis.

<i>Redness, eyelid swelling</i>
0: none
1: mild
2: moderate
3: severe
<i>Chemosis</i>
0: none
1: mild, detectable with slit lamp, conjunctiva separated from sclera
2: moderate (visual evident, raised conjunctiva, especially at the limbal area)
3: severe (ballooning of conjunctiva)
<i>Tearing</i>
0: none
1: mild (eyes feel slightly watery)
2: moderate (blows nose occasionally)
3: severe (tears rolling down cheeks)
<i>Itching (to be graded by subject)</i>
0: none
1: mild (intermittent tickling sensation)
2: moderate (continual awareness but without the desire to rub the eyes)
3: severe (continual awareness with the desire to rub the eyes)
4: incapacitating itching (subject insist on rubbing eyes)

Data from Ref. (7).

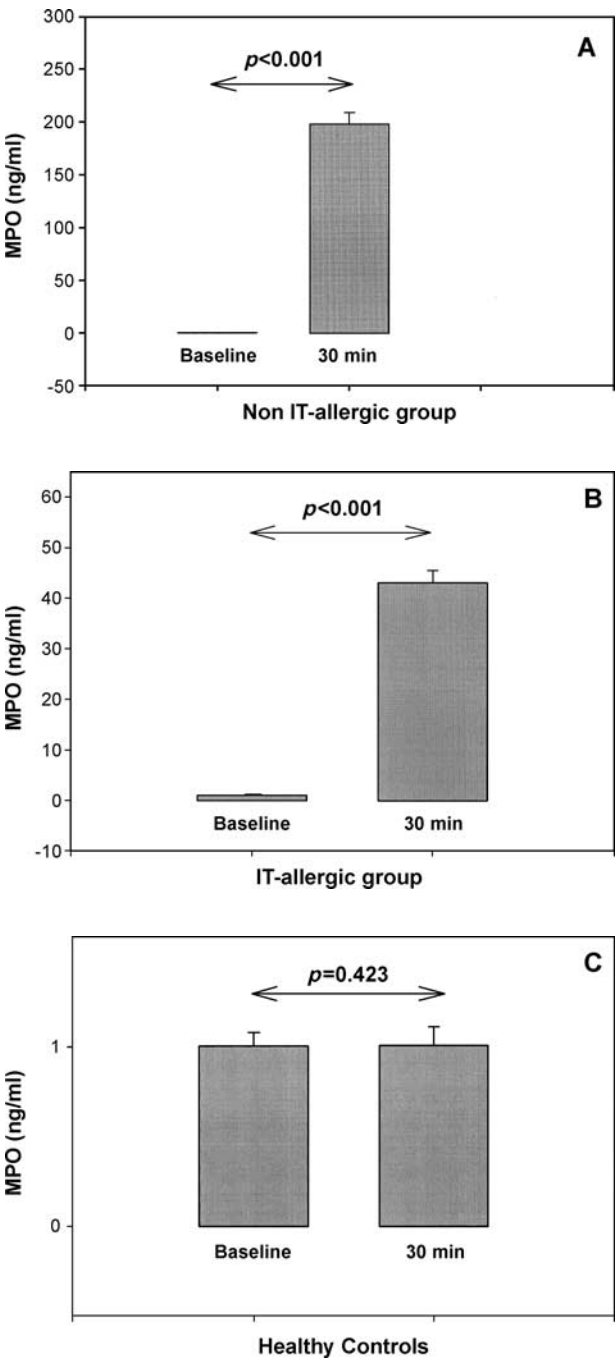


Figure 1. Thirty minutes after challenge, a statistically significant MPO release occurred in the non-IT allergic group (A) and in the IT-allergic group (B) when compared with baseline conditions. Healthy volunteers did not evidence significant MPO release following ASCC (C).

MPO Assay

The MPO activity was measured by MPO-enzyme immunoassay (EIA) method (Oxis International Inc., Portland, OR, USA).

Statistical Analysis

Student's *t* test for independent samples compared mean values. In intragroup comparisons before and after treatment, Student's *t*-test for paired data was used. A *p* value <0.05 was considered significant.

RESULTS

Thirty minutes after challenge, a statistically significant MPO release occurred in the non-IT allergic group ($p<0.001$; Fig. 1A) and the IT allergic group ($p<0.001$; Fig. 1B) when compared with baseline conditions. Neither clinical signs nor MPO release occurred in the contralateral eye challenged with diluent (data not shown). No healthy volunteer evidence any MPO release upon ASCC at the highest allergen dose tested ($p=0.423$; Fig. 1C) in agreement with previous reports (3). Figure 2 shows that the myeloperoxidase levels released in the immunotherapy allergic group were significantly lower than those in the non-immunotherapy allergic group ($p<0.001$) and higher than those in nonallergic subjects ($p<0.001$). The patients of the immunotherapy asthmatic group were checked before and after immunotherapy. Patients of the immunotherapy asthmatic group generated significantly more MPO before than after immunotherapy ($p<0.001$; Fig. 3).

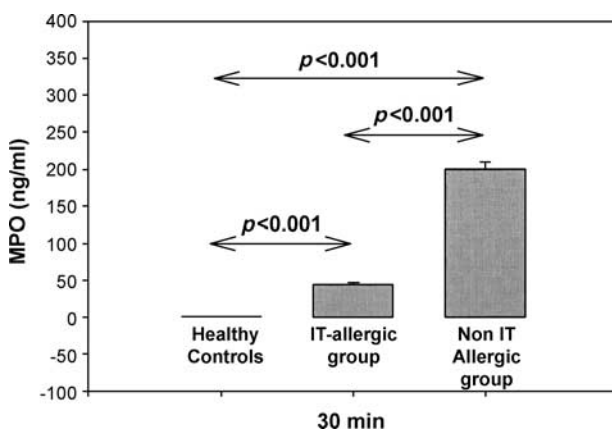


Figure 2. The myeloperoxidase levels released in the immunotherapy allergic group were significantly lower than those in the non-immunotherapy allergic group ($p<0.001$) and higher than those in non-allergic subjects ($p<0.001$).

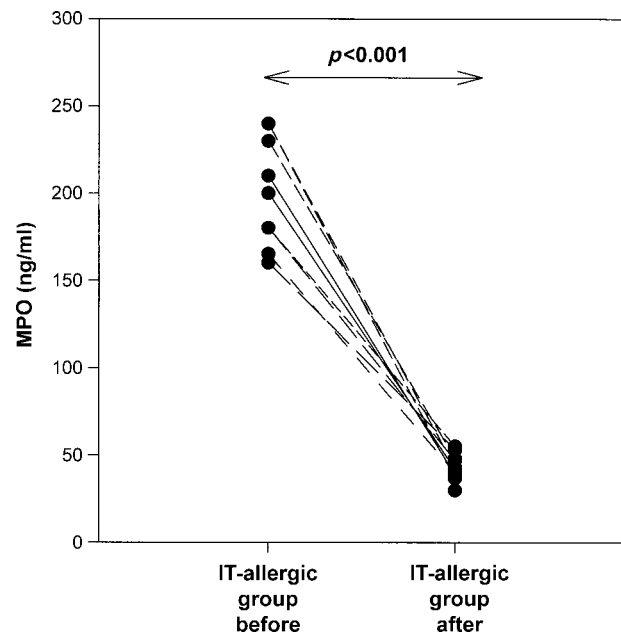


Figure 3. The patients of the immunotherapy-asthmatic group were checked before and after immunotherapy. Patients of the immunotherapy asthmatic group generated significantly more MPO before than after immunotherapy ($p<0.001$).

DISCUSSION

Different authors (9–11) have demonstrated IgE-dependent activation of human neutrophils. The primary (azurophilic) granules of neutrophils contain a variety of enzymes that might potentiate inflammation, such as MPO. MPO is present in different allergic conditions. Neutrophils from patients with asthma have been shown to release higher amounts of MPO than did cells from control subjects (12). In induced sputum, MPO levels were higher in patients with asthma than in control subjects, indicating degranulation of the primary neutrophil granules taking place in the asthmatic condition (13). Allergen challenge of allergic patients produced significantly increased MPO levels in nasal lavage fluid. In contrast, allergen challenge of nonallergic control individuals produced no such response (14,15). MPO was significantly increased in vernal and atopic keratoconjunctivitis (16). Despite such evidence, it is not known whether ASCC is able to elicit MPO release. The experiments described here provide evidence that 30 minutes after challenge, a statistically significant MPO release occurred in the non-IT allergic group when compared with baseline conditions and with levels in the healthy group. We also demonstrate that allergen-immunotherapy treatment of individuals suffering from allergic rhinoconjunctivitis was associated with a significant reduction

in MPO release. Immunotherapy reduces MPO release from neutrophils to levels that approximate those in non-allergic subjects. Previously, other investigators have shown that immunotherapy modifies the functions of neutrophils from asthmatic patients (17). In conclusion, this study provides information for a better understanding of the role of MPO in allergic eye diseases, and suggests that the measurement of MPO in tears may have clinical relevance for the diagnosis and monitoring of these common pathologic conditions

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