

IgE-dependent release of myeloperoxidase by neutrophils from allergic patients

J. MONTESEIRÍN*, I. BONILLA*, M. J. CAMACHO*, J. CONDE*, and F. SOBRINO*†

*Departamento de Medicina, Servicio Regional de Inmunología y Alergia, Hospital Universitario Virgen Macarena and

†Departamento de Bioquímica Médica, Facultad de Medicina, Universidad de Sevilla, Spain

Summary

Background The three forms of IgE receptor: the heterotrimeric high-affinity receptor for IgE (FcεRI), the low-affinity receptor for IgE (FcεRII/CD23) and the Mac-2/IgE-binding protein (εBP), have previously been found on human neutrophils. We have previously shown that specific allergens are able to activate functional responses by neutrophils from allergic patients sensitized to those allergens. Neutrophils are present in the sites of allergic inflammation. The primary (azurophilic) granules of neutrophils contain a variety of enzymes that might potentiate inflammation, such as myeloperoxidase (MPO). It is not known whether specific allergens are able to elicit MPO release by neutrophils from allergic patients.

Methods Neutrophils were challenged *in vitro* with the specific allergen that produced clinical symptoms in asthmatic patients. Also, the cells were challenged with allergens that the patients were not sensitive to. Neutrophils from normal subjects were also challenged with allergens.

Results The *in vitro* challenge of neutrophils with allergens that the patients were sensitive to elicited a release of MPO by these cells. The *in vitro* activation of neutrophils was highly allergen-specific, in such a way that allergens other than those accounting for clinical symptoms did not evoke MPO release, and allergens were ineffective on neutrophils from healthy donors.

Conclusion An IgE-dependent mechanism might promote MPO release by neutrophils at allergic sites.

Keywords: Allergy, asthma, neutrophils, mechanism, IgE-dependent, allergen, myeloperoxidase, release, in-vitro, dose-dependent, time-dependent

Clinical and Experimental Allergy, Vol. 31, pp. 889–892. Submitted 7 March 2000; revised 27 September 2000; accepted 10 November 2000.

Introduction

There is increasing evidence of neutrophil participation in asthma and the allergic process. For instance, neutrophils with altered function were demonstrated in blood from asthmatics, both before and after challenge with antigen. These cellular alterations included: increased complement receptor 3 (CD11b) expression, and increased leukotriene

production and generation of oxygen metabolites. In sputum samples or in bronchoalveolar lavage, challenge with antigen induces an inflammatory reaction that generally includes a burst of neutrophil accumulation during the course of the late-phase response [1]. In order to carry out their crucial role in host defence, 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 haemoprotein located within azurophilic granules. When

Correspondence: Dr J. Monteseirín, Asunción 27, 3° Izda, 41011 Sevilla, Spain, E-mail: fmonteseirinm@meditex.es

neutrophils are stimulated, MPO (together with other granule enzymes) is rapidly secreted and detected extracellularly. Previous studies have demonstrated the presence of the three forms of IgE receptor: the heterotrimeric high-affinity receptor for IgE (FcεRI) [2,3], the low-affinity receptor for IgE (FcεRII/CD23) [4], the Mac-2/IgE-binding protein (εBP) [5–7], on human neutrophils. We have previously shown that specific allergens were able to activate functional responses by neutrophils from allergic patients sensitized to allergens of the same type as those which produce clinical allergic symptoms [8]. Despite these evidences, it is not known whether the release of myeloperoxidase by the neutrophils can be modulated directly by an IgE-dependent mechanism. The present work was undertaken to analyze this question. Here, we present evidence that specific allergens were able to release MPO by neutrophils from allergic patients.

Materials and methods

Materials

The allergens studied included D₁ (*Dermatophagoides pteronyssinus*), G₃ (*Dactylis glomerata*), T₉ (*Olea europea*) and W₆ (*Artemisia vulgaris*). They were purchased from Bial-Aristegui (Bilbao, Spain). Other biochemicals were from Sigma (Madrid, Spain) and Merck (Barcelona, Spain).

Patients and controls

The groups that were studied included adult atopic patients with bronchial asthma and healthy adult non-atopic volunteer controls. The asthmatic patients had positive skin prick tests (Bial-Aristegui) and specific IgE (HYTEC 288, Hycor Biomedical Inc., Irvine, CA, USA) to at least one common allergen (house dust mites and pollens). The subjects received neither treatment nor specific hyposensitization. None of them had experienced episodes of asthma for at least 3 months and none had had respiratory tract infections for 4 weeks before blood sampling. The healthy controls had no history of allergy or bronchial symptoms and had negative skin prick test (Bial-Aristegui) and specific IgE (HYTEC 288) to a battery of inhalant allergens (house dust mites, pollens, moulds and animal danders). The Hospital Ethic Committee approved the study and each subject gave informed consent.

Preparation of polymorphonuclear leucocytes

Human neutrophils were purified from freshly drawn heparinized (10 U/mL) venous blood [8,9]. Neutrophils were resuspended to about 10⁷ cells/mL in phosphate buffered saline (PBS) and 10 mM of glucose and kept at

20°C. These cells were >96% neutrophils as evaluated by morphological analysis. Eosinophil contamination was ≤2%. Trypan blue exclusion showed greater than 96% viability in neutrophils.

Neutrophil stimulation

Cells, resuspended at 1 × 10⁶ in PBS buffer supplemented with 10 mM glucose and 500 μM CaCl₂, were stimulated with a dose of 10 μg/mL of allergens [9] at 37 °C for 45 min. MPO, released with fMLP (Sigma) in a dose of 1 μM at 37 °C for 45 min, was the positive control [10].

MPO assay

After stimulation with allergens or N-Formyl-methionyl-leucyl-phenylalanine (fMLP), the cell suspension was immediately placed on ice to avoid further release and centrifuged at 650 g for 5 min. The MPO activity in the collected supernatant was measured by MPO-EIA method (Oxis International Inc., Portland, OR, USA).

Statistical analysis

Student's unpaired *t*-test or one-way ANOVA made comparisons between groups. A *P*-value < 0.05 was considered significant.

Results

Figure 1(a) illustrates that the treatment of neutrophil with G₃ allergen did elicit the release of MPO by neutrophils obtained from patients who were specifically allergic to G₃ pollen, in both a dose- and time-dependent fashion. Similar results were obtained with other allergens, including *Dermatophagoides pteronyssinus*, *Olea europea*, and *Artemisia vulgaris* (data not shown). Figure 1(b) shows the MPO release taking place when these neutrophils were exposed to fMLP, which was used as positive control, as well as the levels of MPO released by unstimulated cells. Figure 2 illustrates the specificity of the response to allergens. In the allergen-stimulated neutrophils, the cells from the group of asthmatics stimulated with the allergen to which they are sensitive showed significantly higher production of MPO than the cells from healthy donors and the cells from asthmatic patients incubated with an allergen to which the patients were not sensitized (*P* < 0.001). No statistical differences were observed between the levels of MPO released by unstimulated cells and the MPO released by cells from healthy donors and by cells from allergic subjects non-sensitized to the particular allergen.

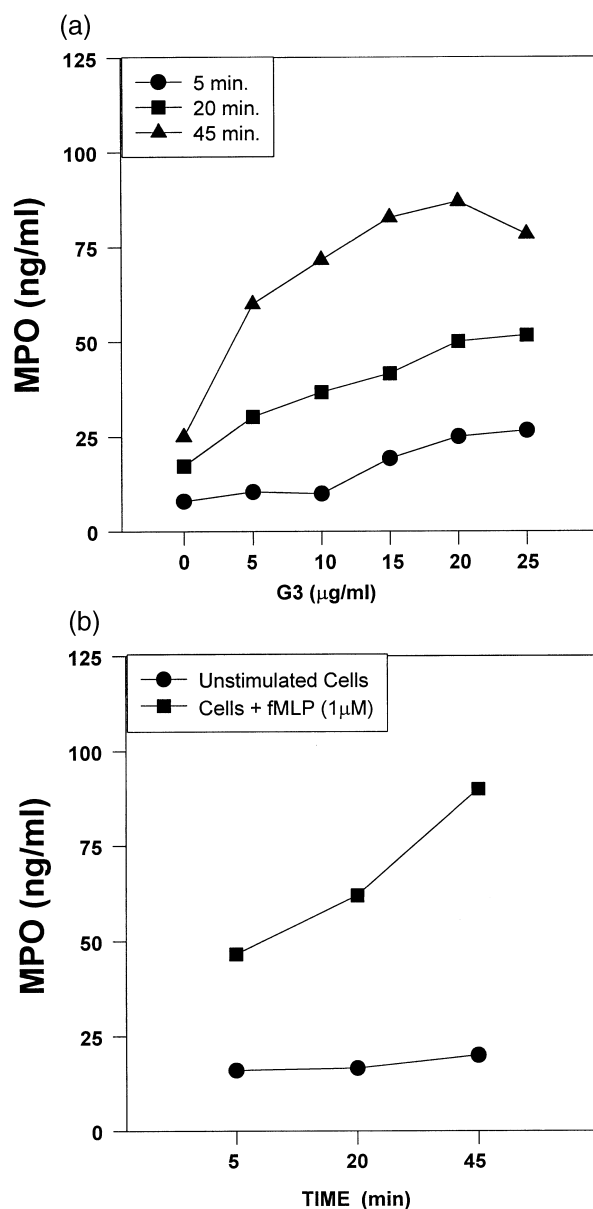


Fig. 1. (a) Challenge with G₃ allergen of neutrophils from asthmatic patients ($n = 12$) who were specifically allergic to G₃ pollen elicited the release of MPO in a dose- and a time-dependent manner. (b) Levels of MPO from neutrophils challenged with fMLP were taken as positive controls. Also, levels of MPO from unstimulated cells are shown.

Discussion

Previous studies have demonstrated the presence of the three forms of IgE receptor on human neutrophils [2–7]. We have previously shown that specific allergens were able to activate functional responses by neutrophils from allergic patients sensitized to allergens of the same type as those which produce clinical allergic symptoms [8]. Neutrophils

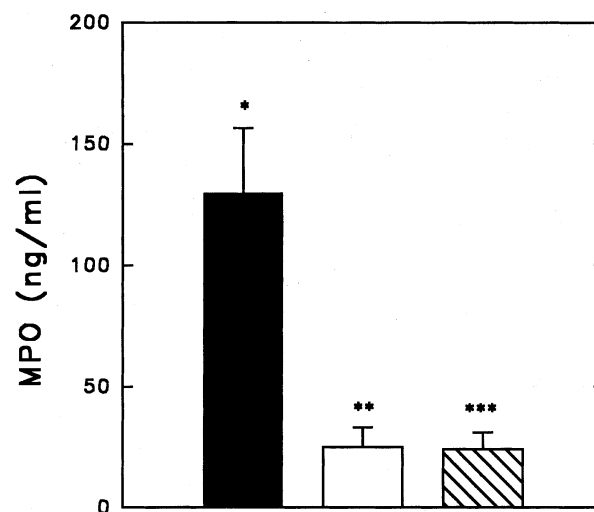


Fig. 2. In the allergen-stimulated neutrophils, the group of asthmatics stimulated with the allergen to which they are sensitive (*) showed a significantly higher production of MPO than cells from healthy donors ($n = 5$) (**) and cells from asthmatic patients ($n = 5$) incubated with an allergen to which they were not sensitized (***) ($P < 0.001$). No statistical differences were observed between the levels of MPO released by unstimulated cells and the MPO released by cells from healthy donors and by cells from allergic subjects non-sensitized to the particular allergen.

are present in the sites of allergic inflammation [1]. The primary (azurophilic) granules of neutrophils contain a variety of enzymes that might potentiate inflammation, such as MPO. Neutrophils from patients with asthma have been shown to release higher amounts of MPO than cells from controls subjects [11]. In induced sputum, MPO levels were elevated in patients with asthma, as compared with control subjects, indicating degranulation of the primary neutrophil granules taking place in the asthmatic condition [12]. Allergen challenge of allergic patients produced a significant late-phase increase in the levels of MPO in nasal lavage fluid. In contrast, allergen challenge of non-allergic control individuals produced no such response [13,14]. Despite this evidence, it is not known whether the release of MPO by the neutrophils from allergic patients can be initiated directly by an IgE-dependent mechanism. The experiments described here provide the first evidence that neutrophils from allergic patients release MPO by an IgE-dependent mechanism. The *in vitro* challenge of neutrophils with allergens to which the patients were sensitive produce a release of MPO dose- and time-dependent fashion. Our results indicate that *in vitro* activation of neutrophils was highly allergen-specific. In this context, allergens other than those which produce clinical symptoms did not elicit MPO release, and allergens were ineffective on neutrophils from healthy donors. However,

neutrophils from both allergic patients and control individuals were able to release MPO upon treatment with fMLP. It follows that an allergen-dependent mechanism might account for a direct release of MPO by neutrophils into allergic sites.

Acknowledgements

This work resulted from equal contributions by the first two authors. This work was supported by grants from Fondo de Investigación Sanitaria (FIS); nos. 97/207 given to J.C.; 94/1484 and 97/1289 given to F.S.; from Foundation of SEAIC given to J.M.; from Bial-Aristegui and by the Junta de Andalucía (Ayudas Grupos de Investigación) of Spain and Hycor Biomedical Inc., USA.

References

- 1 Henson PM, Borish LC. Neutrophils mediators in asthma. In: Busse WW, Holgate ST, eds. *Asthma and rhinitis*. Boston: Blackwell Scientific Publications, 1995:367–82.
- 2 Scholl PR, Geha RS. Physical association between the high-affinity IgG receptor (FcγRI) and the subunit of the high-affinity IgE receptor (FcεRI). *Proc Natl Acad Sci USA* 1993; 90:8847–50.
- 3 Soussi-Gounni A, Lamkhieoued B, Christodoulouopoulos P, Nutku E, Hamid Q. Human neutrophils express the high affinity receptor for IgE (FcεRI). *J Allergy Clin Immunol* 1998; 101:S173.
- 4 Yamaoka KA, Arock M, Issaly F, Dugas N, Le Goff L, Kolb JP. Granulocyte macrophage colony stimulating factor induces Fc epsilon RII/CD23 expression on normal human polymorphonuclear neutrophils. *Int Immunol* 1996; 8:479–90.
- 5 Truong MJ, Gruart V, Kusnierz JP et al. Human neutrophils express immunoglobulin E (IgE)-binding proteins (Mac2/εBP) of the S-type lectin family: Role in IgE-dependent activation. *J Exp Med* 1993; 117:243–8.
- 6 Yamaoka A, Kuwabara I, Frigeri LG, Liu FT. A human lectin, galectin-3 (εBP/Mac2), stimulates superoxide production by neutrophils. *J Immunol* 1995; 154:3479–87.
- 7 Liu FT, Hsu DK, Zuberi RI et al. Modulation of functional properties of galectin-3 by monoclonal antibodies binding to the non-lectin domains. *Biochemistry* 1996; 14:6073–9.
- 8 Monteseirín J, Camacho MJ, Montaña R et al. Enhancement of antigen-specific functional responses by neutrophils from allergic patients. *J Exp Med* 1996; 183:2571–9.
- 9 Carballo M, Marquez G, Conde M et al. Characterization of calcineurin in human neutrophils. *J Biol Chem* 1999; 274:93–100.
- 10 Sengeløv H, Follin P, Kjeldsen L, Lollike K, Dahlgren C, Borregaard N. Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. *J Immunol* 1995; 154:4157–65.
- 11 Carlson M, Håkansson L, Peterson Ch, Stålenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J Allergy Clin Immunol* 1991; 87:27–33.
- 12 Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med* 1997; 155:449–53.
- 13 Jacobi HH, Poulsen LK, Reimert CM et al. IL-8 and the activation of eosinophils and neutrophils following nasal allergen challenge. *Int Arch Allergy Immunol* 1998; 116:53–9.
- 14 Palczynski C, Krakowiak A, Ruta U et al. Nasal response to allergen challenge in patients with immediate asthmatic reaction. *Allergol Immunopathol* 1996; 24:163–8.