

Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: The effect of immunotherapy

Javier Monteseirín, MD, PhD,^a Inés Bonilla, MD, PhD,^a Jesús Camacho, MD, PhD,^a
José Conde, MD, PhD,^a and Francisco Sobrino, PhD^{a,b} *Seville, Spain*

Background: There is increasing evidence of neutrophil participation in asthma and the allergic process. After activation, neutrophils release myeloperoxidase (MPO) together with other granule enzymes.

Objectives: In this study we attempted to evaluate the release of MPO in vitro by neutrophils from asthmatic patients and the relationship between neutrophil degranulation and lung function, measured as FEV₁, of the patients. We also investigated the possible role of immunotherapy in the release of MPO by neutrophils.

Methods: Neutrophils were stimulated with formyl-methionyl-leucyl-phenylalanine for 45 minutes at 37°C. MPO released from neutrophils was assayed by using an MPO enzyme immunoassay.

Results: Neutrophils released statistically significantly higher MPO levels in the asthmatic patients not receiving immunotherapy than in the healthy group. A significant inverse correlation was observed in the asthmatic group not receiving immunotherapy between MPO secretion and lung function, measured as FEV₁, of the patients. Neutrophils of the asthmatic group receiving immunotherapy released significantly less MPO than did those of the asthmatic group not receiving immunotherapy, with MPO levels equal to those from nonallergic subjects.

Conclusions: We conclude that neutrophils obtained from allergic asthmatic patients have an increased propensity to release MPO. The experiments described here provide evidence that there is a significant inverse relationship between levels of MPO released by neutrophils from allergic patients and lung function, as assessed by FEV₁. Our study suggests that immunotherapy actively modifies the release of MPO in vitro by neutrophils from allergic asthmatic patients. (*J Allergy Clin Immunol* 2001;107:623-6.)

Key words: Allergy, asthma, neutrophils, mechanism, IgE-dependent, allergen, myeloperoxidase, immunotherapy

Abbreviations used

fMLP: N-formyl-methionyl-leucyl-phenylalanine

IT: Immunotherapy

MPO: Myeloperoxidase

Bronchial asthma is characterized by reversible bronchial obstruction with airway inflammation.¹ These inflammatory processes are induced by complex reactions between inflammatory cells, such as neutrophils, and the release of granule proteins.^{2,3} After activation, neutrophils release myeloperoxidase (MPO) together with other granule enzymes. MPO may then react with H₂O₂ generated during the respiratory burst together with a halide (usually Cl⁻) to generate HOCl and other related compounds with wide biologic activity. For example, mast cells may be stimulated to release histamine and cause injury to various cells and tissues both in vivo and in vitro.⁴⁻⁷ On the other hand, in children with bronchial asthma, the release of histamine was significantly correlated with superoxide anion generation, suggesting that a basic intracellular abnormality is related to increased bronchial inflammation.⁸ Furthermore, in another study⁹ superoxide production was measured in the resting state and after stimulation with phorbol myristate acetate, formyl-methionyl-leucyl-phenylalanine (fMLP), or calcium ionophore A23187. Inhibition of this response by histamine was also measured. Neutrophils from atopic subjects produced more superoxide in the basal state or in response to calcium ionophore A23187 or submaximal concentrations of fMLP than did cells from control subjects. Histamine inhibition of fMLP-stimulated superoxide production was less in atopic subjects than in control subjects. These findings suggest that neutrophils from atopic subjects may be hyperreactive in that they produce more superoxide in response to stimuli and are less readily inhibited than cells from control subjects. Such properties may reflect a general attribute of cellular function in atopy and may contribute directly to tissue damage in allergic diseases. In this study we attempted to evaluate the release of MPO in vitro by neutrophils from asthmatic patients and the relationship between neutrophil degranulation and lung function, measured as FEV₁, of the patients. We also investigated the possible role of immunotherapy in the release of MPO by neutrophils.

From ^aDepartamento de Medicina, Servicio Regional de Inmunología y Alergia, Hospital Universitario Virgen Macarena and ^bDepartamento de Bioquímica Médica, Facultad de Medicina, Universidad de Sevilla, Seville.

Supported by grants from the Fondo de Investigación Sanitaria (FIS; No. 97/207 to J. C. and Nos. 94/1484 and 97/1289 to F. S.); from the Foundation of SEAIC given to J. M.; by Bial-Aristegui and the Junta de Andalucía (Ayudas Grupos de Investigación) of Spain; and by Hycor Biomedical, Inc.

Received for publication March 3, 2000; revised December 6, 2000; accepted for publication December 13, 2000.

Reprint requests: Javier Monteseirín, MD, PhD, Asunción 27, 3° Izda, 41011 Seville, Spain.

Copyright © 2001 by Mosby, Inc

0091-6749/2001 \$35.00 + 0 1/81/113566

doi:10.1067/mai.2001.113566

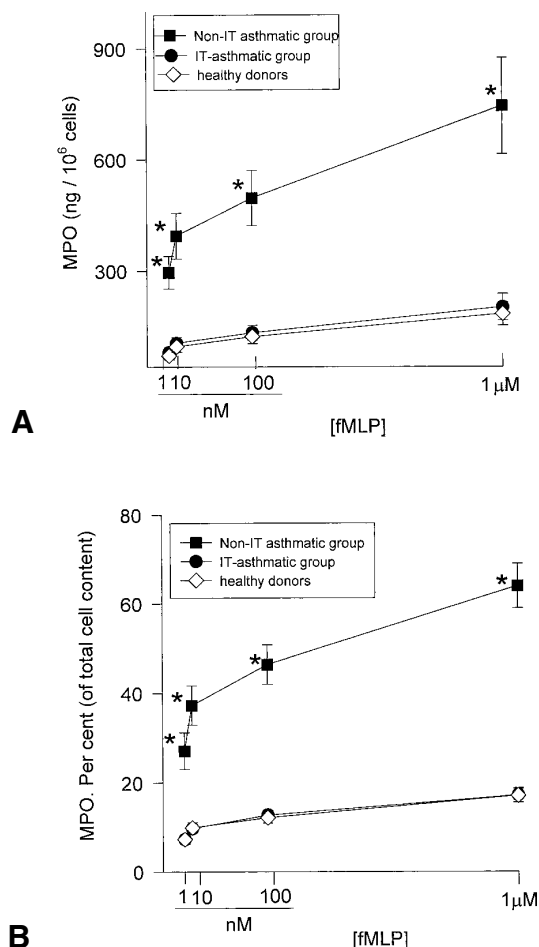


FIG 1. MPO dose-response curve. Neutrophil degranulation was measured by means of MPO release after stimulation with 4 doses of fMLP (1, 10, and 100 nmol/L and 1 μ mol/L). After 45 minutes of incubation, neutrophils from asthmatic patients not receiving immunotherapy (*non-IT asthmatic group*) released significantly higher ($P < .001$) amounts of MPO at all 4 doses of fMLP, expressed both as nanograms per 10^6 cells (**A**) and as release of MPO in percentage of total cell content (**B**), than did neutrophils from the asthmatic group receiving immunotherapy (*IT-asthmatic group*) and neutrophils from healthy donors. Neutrophils from asthmatic patients receiving immunotherapy released MPO levels equal to those from nonallergic subjects. All populations had roughly the same total amount of MPO in unstimulated cells. * $P < .001$.

METHODS

Materials

All biochemicals were obtained from Sigma (Madrid, Spain), Serva (Barcelona, Spain), or Merck (Barcelona, Spain).

Patients and control subjects

The groups studied included adult atopic patients with bronchial asthma and healthy, adult, nonatopic, volunteer control subjects. One group of asthmatic patients ($n = 14$) had positive skin prick test (Bial-Aristegui, Bilbao, Spain) responses and specific IgE (HYTEC 288; Hycor Biomedical, Inc, Irvine, Calif) to at least one common allergen (house dust mites and pollens). The subjects received no specific hyposensitization (ie, asthmatic group not receiving immunotherapy).

Another group of asthmatic patients ($n = 14$) had received immunotherapy with *Dermatophagoides pteronyssinus* extract (Bial-Aristegui) for the preceding 3 years and continued to receive a maintenance dose within the highest potency of the extract (ie, asthmatic group receiving immunotherapy). The patients were not allowed to take any bronchodilators within the 8 hours before challenge of neutrophils in vitro. Oral bronchodilators were withheld for 24 hours, and none of the subjects had taken corticosteroids, cromolyn sodium, or nedocromil sodium in the previous week. The healthy control subjects ($n = 14$) had no history of allergy or bronchial symptoms and had negative skin prick test (Bial-Aristegui) responses and specific IgE (HYTEC 288) to a battery of inhalant allergens (house dust mites, pollens, molds, and animal danders). The Hospital Ethics Committee approved the study, and each subject gave informed consent.

Lung function

FEV₁ was measured with a dry spirometer (Vitalograph, Buckingham, UK). The best value of 3 maneuvers was expressed as a percentage of the predicted value.

Preparation of polymorphonuclear leukocytes

Human neutrophils were purified¹⁰ from fresh-drawn heparinized (10 U/mL) venous blood in 2 steps. First, the blood (20 mL) was mixed with 1.5 mL of 10% Dextran T 500 (final concentration of 0.7%) dissolved in PBS. The supernatant fraction was centrifuged through a Ficoll-Hypaque. The neutrophil-rich pellet was then removed with a plastic pipette, and red cells were eliminated with one hypotonic lysis in water. Neutrophils were resuspended to about 10^7 cells/mL in PBS and 10 mmol/L glucose and kept at room temperature. These cells were greater than 96% neutrophils, as evaluated by morphologic analysis. Eosinophil contamination was 2%. Trypan blue exclusion showed greater than 96% viability in neutrophils.

Neutrophil stimulation with fMLP

Cells resuspended at 1×10^6 in PBS buffer supplemented with 10 mmol/L glucose and 500 μ mol/L CaCl₂ were stimulated with fMLP (1, 10, and 100 nmol/L and 1 μ mol/L)^{11,12} for 45 minutes at 37°C.

MPO assay

MPO released from neutrophils was assayed with an MPO-EIA (Oxis International, Inc, Portland, Ore). After stimulation with fMLP, the cell suspension was immediately placed on ice to avoid further release and centrifuged at 2000 rpm for 5 minutes.

Statistical analysis

Data are expressed as means \pm SEM. Comparisons between groups were made with one-way ANOVA. A P value of less than .05 was considered significant. Regression analysis was performed by using Pearson rank correlation coefficients. A P value of less than .05 was considered significant.

RESULTS

MPO dose-response curve

Neutrophil degranulation was measured by means of MPO release after stimulation with 4 doses of fMLP (1, 10, and 100 nmol/L and 1 μ mol/L).^{11,12} After 45 minutes of incubation, neutrophils from asthmatic patients not receiving immunotherapy released significantly higher ($P < .001$) amounts of MPO at all 4 doses of fMLP, expressed both as nanograms per 10^6 cells (Fig 1, A) and as release of MPO in percentage of total cell content (Fig 1, B), than did neutrophils from the asthmatic group

receiving immunotherapy and neutrophils from healthy volunteers. Neutrophils from asthmatic patients receiving immunotherapy released MPO levels equal to those from nonallergic subjects. All populations had roughly the same total amount of MPO in unstimulated cells ($P = .950$).

Relationship between patients' lung function and degranulation of neutrophils

The amount of MPO released by neutrophils after incubation with $1 \mu\text{mol/L}$ fMLP was evaluated in relation to the patients' lung function, measured as FEV_1 . A significant inverse correlation was observed in the asthmatic group not receiving immunotherapy between the secretion of MPO and lung function of the patients ($r = -0.608$, $P = .021$; Fig 2).

DISCUSSION

Other investigations have demonstrated the relationship of MPO with allergic processes. Thus in induced sputum MPO was elevated in patients with asthma compared with control subjects, indicating degranulation of the primary neutrophil granules in asthma.¹³ 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 nonallergic control subjects produced no such response.¹⁴ Previously, it has been demonstrated, in concordance with our results, that neutrophils stimulated in vitro with serum-opsonized Sephadex particles from patients with asthma released significantly more MPO than did cells from control subjects.¹⁵ Nevertheless, in the article of Carlson et al,¹⁵ there was no relationship between the increased neutrophil degranulation and lung function, measured as peak expiratory flow, of the patients. The reason might be that all patients had been using medication, including β_2 -agonists, glucocorticosteroids, and theophylline, at the time of neutrophil challenge. The experiments described here provide evidence that there is a significant inverse relationship between MPO levels released by neutrophils from allergic patients and lung function, as assessed by FEV_1 . We conclude that neutrophils obtained from allergic asthmatic patients have an increased propensity to release MPO. Dose responses for fMLP in all groups show (Fig 1) that the same concentration of fMLP causes a maximal response. Our results suggest persistent neutrophil activation in asthma.¹⁶ Accumulation of activated neutrophils in the lungs might maintain an inflammatory process, which eventually results in a decrease in FEV_1 . We also demonstrated that treatment of individuals with allergic asthma with allergen immunotherapy was associated with a significant reduction in MPO released in vitro. Immunotherapy reduces MPO released from neutrophils to levels equal to those from nonallergic subjects. Other investigators have shown the effect of immunotherapy on the functions of neutrophils from asthmatic patients. During the high-load pollen season, they found a significantly higher neutrophil chemotactic activity in bronchoalveolar lavage fluid in untreated

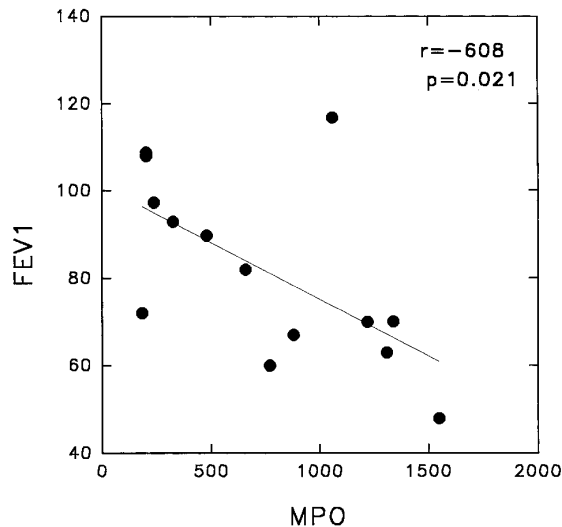


FIG 2. Relationship between the patients' lung function and the degranulation of neutrophils. The amount of MPO released by neutrophils after incubation with $1 \mu\text{mol/L}$ fMLP was evaluated in relation to the patients' lung function, measured as FEV_1 . Significant inverse correlation was observed in the asthmatic group not receiving immunotherapy between the secretion of MPO and lung function of the patients ($r = -0.608$, $P = .021$).

patients than in immunotherapy-treated patients.¹⁷ Our study suggests that immunotherapy actively modifies the release of MPO in vitro from neutrophils from allergic asthmatic patients; however, a prospective placebo-controlled study examining MPO production in neutrophils during and after immunotherapy is necessary to confirm our results.

In conclusion, neutrophils obtained from allergic asthmatic patients have an increased propensity to release MPO. Our results suggest persistent neutrophil activation in asthma and may indicate that these cells could have been primed by allergens, as we have demonstrated previously. There is a significant inverse relationship between levels of MPO released by neutrophils from allergic patients and lung function as assessed by FEV_1 . Finally, immunotherapy actively modifies the release of MPO in vitro by neutrophils from allergic asthmatic patients.

This work is the result of an equal contribution of the first 2 authors.

REFERENCES

1. Boushey HA, Holzman MJ, Sheller MJ, Nadel JA. Bronchial hyperreactivity. *Am Rev Respir Dis* 1980;121:389-413.
2. Chung KF. Role of inflammation in the hyperactivity of the airways in asthma. *Thorax* 1986;41:657-62.
3. Venge P. Soluble markers of allergic inflammation. *Allergy* 1994;49:1-8.
4. Klebanoff SJ. Oxygen-dependent cytotoxic mechanisms of phagocytes. In: Gallin JI, Fauci AS, eds. *Advances in host defence mechanisms*. New York: Raven Press; 1982. p. 111-62.
5. Henderson WR, Chi EY, Klebanoff SJ. Eosinophil peroxidase-induced mast cell secretion. *J Exp Med* 1980;152:265-79.
6. Shasby DM, Vanbenthuysen KM, Tate RM, Shasby SS, McMurtry I, Respine JE. Granulocytes mediate acute edematous lung injury in rabbits

- and in isolated rabbit lungs perfused with phorbol myristate acetate: role of oxygen radicals. *Am Rev Respir Dis* 1982;125:443-7.
7. Petrone WF, English DK, Wong K, McCord JM. Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. *Proc Natl Acad Sci U S A* 1980;77:1159-63.
 8. Neijens HJ, Raatgeep RE, Degenhart HJ, Diuerman EJ, Kerrebijn KF. Altered leukocyte response in relation to the basic abnormality in children with asthma and bronchial hyperresponsiveness. *Am Rev Respir Dis* 1984;130:744-7.
 9. Styrt B, Rocklin RE, Klempner MS. Characterization of the neutrophil respiratory burst in atopy. *J Allergy Clin Immunol* 1988;81:20-6.
 10. Böyum A. Isolation of mononuclear cells and granulocytes from blood. *Scand J Clin Lab Invest* 1986;97:77-89.
 11. Dang Y, Lowe GM, Edwards SW, Galvani DW. The effects of GM-CSF on myeloperoxidase release in normal and myelodysplastic neutrophils. *Leukemia Res* 1993;17:1037-44.
 12. Sullivan GW, Carper HT, Mandell GL. The effect of three human recombinant hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and interleukin-3) on phagocyte oxidative activity. *Blood* 1993;81:1863-70.
 13. Bretz U, Baggiolini M. Biochemical and morphological characterization of azurophil and specific granules of human neutrophilic polymorphonuclear leukocytes. *J Cell Biol* 1974;63:251-8.
 14. Jacobi HH, Poulsen LK, Reimert CM, Skov PS, Ulfgren AK, Jones I, et al. IL-8 and the activation of eosinophils and neutrophils following nasal allergen challenge. *Int Arch Allergy Immunol* 1998;116:53-9.
 15. 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.
 16. Monteseirín J, Camacho MJ, Montaña R, Llamas E, Conde M, Carballo M, et al. Enhancement of antigen-specific functional responses by neutrophils from allergic patients. *J Exp Med* 1996;183:2571-9.
 17. Rak S, Björnson A, Håkanson L, Sörenson S, Venge P. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J Allergy Clin Immunol* 1991;88:878-88.

Receive tables of contents by e-mail

To receive the tables of contents by e-mail, sign up through our Web site at

<http://www.mosby.com/jaci>

Choose *E-mail Notification*

Simply type your e-mail address in the box and click the *Subscribe* button

Alternatively, you may send an e-mail message to majordomo@mosby.com.

Leave the subject line blank and type the following as the body of your message:

subscribe jaci_toc

You will receive an e-mail message confirming that you have been added to the mailing list.

Note that TOC e-mails will be sent out when a new issue is posted to the Web site.