

Original Article

Defective neutrophil function in patients with perennial allergic rhinitis

Popi Lianou, Athanasios Fortis, Nikolaos Papadopoulos, Nikolaos Trakas and John Papavassiliou

Center of Immunology for Infectious Diseases, Department of Microbiology, Medical School, University of Athens, Athens, Greece

ABSTRACT

In the present study, neutrophil functions were examined *in vitro* in 35 patients (15 men and 20 women; mean age 38 years) suffering from perennial allergic rhinitis. Adherence to nylon, serum chemotaxis and phagocytosis–bactericidal activity were assayed. A *Candida albicans* strain was used for the experiments of phagocytosis–bactericidal activity. Adherence to patients' neutrophils was found in normal patients ($22.6 \pm 1.89\%$) and healthy controls ($26.1 \pm 1.59\%$; $P > 0.05$). In contrast, the chemotactic ability of the patients' sera was inferior to that of healthy control patients (leucotactic index 20.25 ± 0.76 vs $28.04 \pm 0.46 \mu\text{m}$, respectively; $P < 0.0001$). Patients' neutrophils phagocytosed 102.77 ± 6.03 microorganisms per 100 cells, while controls phagocytosed 138.05 ± 7.60 microorganisms per 100 cells ($P < 0.001$). Bactericidal activity was also severely impaired in patients compared with controls (17.19 ± 1.67 vs $33.23 \pm 1.96\%$, respectively; $P < 0.0001$). The observed host defense disorders provide additional information that helps to explain the increased susceptibility of these patients to bacterial infections.

Key words: atopy, host defense, neutrophils, perennial allergic rhinitis.

INTRODUCTION

Allergic rhinitis is common. It is mainly found in atopic individuals. Between 10 and 20% of the US population is affected.¹ Perennial allergic rhinitis (PAR) is due to allergens that are present throughout the year. Perennial nasal symptoms indicate chronic allergy due to indoor factors.

Numerous studies have been performed over the past few years investigating the interrelationship of host defense mechanisms and atopy. However, evidence of neutrophil involvement in allergic rhinitis has been circumstantial.² Nevertheless, there is evidence that there is an impairment of neutrophil function in PAR patients. Neutropenic subjects suffer from chronic (perennial) rhinitis 3.4-fold more frequently than non-neutropenics.³ Other authors consider that persistent inflammation may further lead to the dysregulation of local cellular immunity by reducing the number and activity of neutrophils on the mucosal surface.⁴

Secondary infections of sinuses and the middle ear are commonly found in this type of allergic rhinitis.^{5,6} It is not yet known whether atopy itself affects the function of neutrophils as a result of the participation of neutrophils in a chronic inflammatory procedure, rendering patients more susceptible to infections, or whether infections occur only on the basis of anatomic and physiologic alterations caused by allergic rhinitis. The study of neutrophils in atopic subjects is potentially important for at least two reasons. First, the neutrophil is used as an available model cell, in which general characteristics of tissue responsiveness can be assessed. Second, the release of toxic substances by neutrophils may contribute to the morbidity of allergic disease.⁷ Therefore, in the present study, we examined neutrophil functions in patients suffering from PAR without any other health problem and who had no symptoms when they were tested.

Correspondence: Dr AA Fortis, Kerkis 71 St, Petroupoli 13231, Athens, Greece. Email: at40s@hotmail.com

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METHODS

Patients

Thirty-five patients (15 men and 20 women; mean age 38 years, range 21–54 years) suffering from PAR, with no other health problems, were included in the study. Diagnosis was based on history data and on clinical findings, as well as a positive response to intradermal skin tests (patients were allergic to mite allergens). One month before the study and during the study, there were no symptoms and no medication was administered. There was not any clinical or laboratory evidence of infection for the same period. All patients had reported frequent episodes of atopic symptomatology in the past.

The comparison group consisted of 35 healthy volunteers paired for age (mean 38 years, range 20–58 years) and sex (15 men and 20 women).

Neutrophil preparation

Heparinized blood was mixed with 6% dextran in 0.15% mol/L NaCl. Erythrocytes were sedimented at room temperature for 45 min. The leukocyte-rich supernatant was centrifuged to obtain a pellet of leukocytes. This was washed twice in RPMI medium 1640 (Gibco, Grand Island, NY, USA) and layered onto Ficoll-Hypaque (Pharmacia, Piscataway, NJ, USA) gradient. Following separation, polymorphonuclear leukocytes were suspended in the same medium at a concentration of approximately 10 000 cells/mL.⁸ By microscopy, the preparation contained less than 5% mononuclear cell contamination and neutrophils made up more than 90% of the sample. Viability exceeded 98% as assessed by trypan blue dye exclusion. The percentage of eosinophils in our patients when the tests were performed was $6 \pm 2\%$, while in the healthy controls it was $2 \pm 2\%$.

Adherence to nylon

Adherence of neutrophils to nylon was assayed by a minor modification of the method of McGregor *et al.*⁹ Briefly, 60 mg 12 mm columns of nylon fiber (3 denier, 4 cm; type 200; Fenwal Laboratories, Morton Groove, IL, USA) were constructed in Pasteur pipettes. The fiber was packed by means of a wooden applicator stick in the pipette. The columns were not washed or flushed with any fluid before use. Aliquots (1 mL) of the neutrophil suspension were filtered through the three columns, which had been warmed previously to 37°C in an incubator.

Adherence was calculated by averaging the percentage of neutrophils retained in these columns.

Chemotaxis

The chemotactic assay was performed with the modified acrylic Boyden chambers (Alcho, Southington, CT, USA), as described previously.¹⁰ Boyden chambers consist of two compartments, the upper and lower, separated by a micropore filter (MF filters; Millipore, Bedford, MA, USA). The neutrophil suspension is placed in the upper compartment and the chemoattractant in the lower. The chamber is incubated for 90 min at 37°C and the filter is then removed, fixed and stained with hematoxylin. To evaluate the results, cells are counted at 10 µm intervals starting from the proximal filter surface and the average distance traveled by cells (leucotactic index, LI) is calculated. In our experiments, the chemoattractive ability of the subjects' sera was tested on normal neutrophils derived from a young healthy volunteer. Serum was activated by the addition of 5 mg/mL Zymosan (Sigma Chemical Co., St Louis, MO, USA) and subsequent agitation for 30 min at 37°C. Control experiments in which no chemoattractant was used were run concurrently.

Phagocytosis and bactericidal function

The phagocytic and bactericidal functions of neutrophils were assayed as described by Smith and Rommel.¹¹ *Candida albicans* was selected as the test microorganism. The strain used was isolated from urine cultures taken from patients with asymptomatic urinary tract infection and was sensitive to the usual antibiotics. *Candida albicans* was harvested after three days' culture in Sabouraud medium. Microorganisms were suspended in 10 mL phosphate-buffered saline (PBS) and washed three times with PBS by centrifugation and then resuspended in PBS at a concentration of 10⁶ microorganisms/mL, as determined spectrophotometrically. Six drops of freshly drawn non-anticoagulated whole peripheral venous blood were placed on a glass coverslip and incubated in a humidity chamber at 37°C. After 45 min, the clot was removed and the glass coverslip was gently rinsed with RPMI 1640. The glass coverslip with the adherent phagocytes was then flooded with 1.8 mL bacterial suspension and 0.2 mL autologous serum, followed by a second incubation at 37°C for 45 min. Finally, the glass coverslip was stained with acridine orange fluorochrome stain (Fisher Scientific, Springfield, NJ, USA). Stained viable bacteria

appear green, whereas non-viable bacteria appear red when viewed by fluorescence microscopy. Adhering bacteria can be distinguished from phagocytosed bacteria because they lose their fluorescence with crystal violet stain. Two different glass coverslips for each subject were assayed; 50 phagocytic cells were counted each time. The mean number of phagocytosed bacteria per cell and the percentage of non-viable to phagocytosed bacteria was then calculated.

Statistical analysis

Data were analyzed using two-tailed Student's *t*-test. Due to the fluctuation of the phagocytic activity of the neutrophils of various individuals, a paired *t*-test was used for the phagocytosis assay.

RESULTS

Adherence

There were no pathologic findings relative to the adherence of neutrophils to nylon. The mean (\pm SEM) adherence of the patients' neutrophils was $22.6 \pm 1.89\%$ compared with $26.1 \pm 1.59\%$ for the control group ($P > 0.05$; Fig. 1).

Serum chemotaxis

When Zymosan-activated serum from the control group was used as the chemoattractant, the mean distance traveled by neutrophils (LI) was $28.04 \pm 0.46 \mu\text{m}$, whereas in the case of the patients the LI was $20.25 \pm 0.76 \mu\text{m}$. These results demonstrate that patients' sera had a significantly lower chemotactic effect on normal neutrophils ($P < 0.001$; Fig. 2).

Phagocytosis

The phagocytic ability of the patients' neutrophils was severely impaired compared with that of healthy controls. The mean number of bacteria ingested per neutrophil in the control group was 138.05 ± 7.6 per 100 cells, while patients' neutrophils ingested 102.77 ± 6.03 microorganisms per 100 cells ($P < 0.001$; Fig. 3).

Bactericidal function

The percentage of ingested bacteria killed by the neutrophils was also reduced in the patient group. Neutrophils

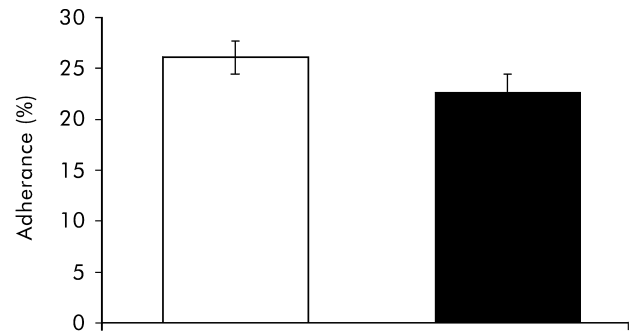


Fig. 1 Percentage of neutrophils adhering to nylon fibers in healthy subjects (□) and patients (■).

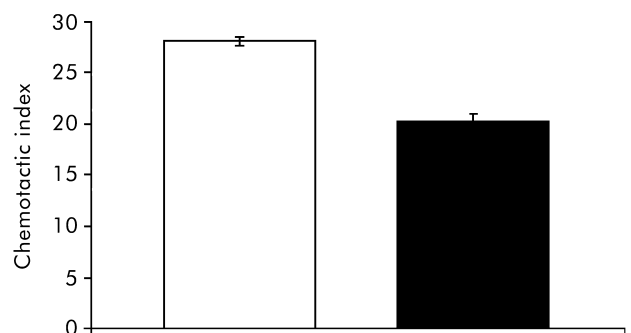


Fig. 2 Neutrophil chemotactic index in controls (□) and patients (■).

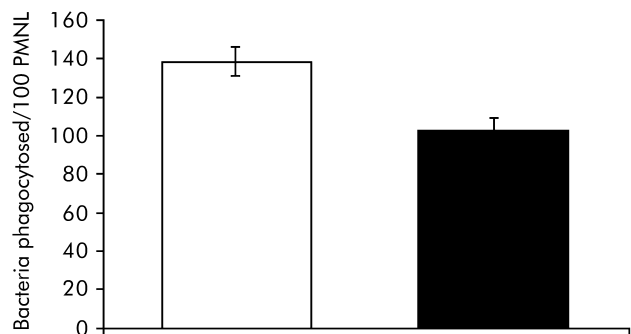


Fig. 3 Neutrophil phagocytosis in controls (□) and patients (■).

from the control group killed $33.23 \pm 1.96\%$ of *C. albicans*, whereas neutrophils derived from patients killed $17.19 \pm 1.67\%$ ($P < 0.001$; Fig. 4).

DISCUSSION

Almost everyone agrees that atopic conditions, such as allergic rhinitis and asthma, include an inflammatory process. The main function of the neutrophils is the

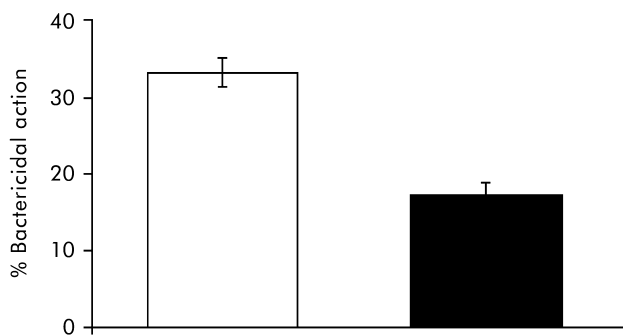


Fig. 4 Percentage of bacteria killed by neutrophils (bactericidal action %) in controls (□) and patients (■).

support of non-specific host defense mechanisms. The inflammatory response during allergic airway inflammation involves the recruitment of multiple leukocyte populations, which includes neutrophils, monocytes, lymphocytes and eosinophils. All these populations likely contribute to the pathology observed during repeated episodes of allergic inflammation.¹² The extent of the inflammatory reaction determines the seriousness of the allergic disease.¹³

Our results demonstrate that adherence to nylon of the peripheral blood neutrophils from patients with PAR who were in a quiescent (symptomless) interval was normal. The regulation of epithelial cell adhesion molecules is considered to be cytokine orchestrated in both seasonal allergic rhinitis and PAR.¹⁴ Intercellular adhesion molecule (ICAM)-1 expression on epithelial cells occurs as an early event (30 min after allergen-specific challenge) and persists up to 48 h.¹⁵ Moreover, Frew *et al.* have established that, in asthmatics, there was no significant endothelial expression after local endobronchial allergen challenge of P-selectin, E-selectin, ICAM-1 or vascular cell adhesion molecule-1 when 24 h had elapsed. There was also no significant difference in neutrophils in the submucosa.¹⁶

The identity of the migrating leukocytes in an inflammatory procedure varies depending on the nature of the inciting stimulus and also changes as the inflammatory site ages (neutrophils predominate for the first 6–24 h).¹⁷ According to the mentioned above, we assume that the interpretation of normal adherence is based on the fact that our experiments took place when patients were in a latent phase, with no exacerbation of the inflammatory process.

Studies regarding increased chemotactic activity of neutrophils in allergic rhinitis and other atopic diseases

were performed after allergen challenge of subjects or when nasal symptoms were present.^{18,19} Therefore, the relevance of patients who are in a quiescent (symptomless) interval is uncertain. In contrast, impairment of neutrophilic chemotaxis has been reported in patients with other atopic disorders.²⁰ Hakanson *et al.* studied the formation of heat-stable and heat-labile neutrophil chemotactic activity in serum after allergen challenge in allergic subjects during and after the pollen season. Both heat-stable and heat-labile activity was significantly reduced after the pollen season compared with that before the pollen season. The authors suggested the generation of an inhibitor at this period.²¹ Our serum chemotaxis experiments, which were performed with normal neutrophils, indicate that the patients' serum is either less functional or contains some type of inhibitor in accordance with the inhibitor proposed by Hakanson *et al.*

Our data also suggest a defective phagocytosis and bactericidal function of neutrophils.

Untreated seasonal allergic rhinitis patients have less neutrophil phagocytic activity and a decreased count of Fc receptors.²² Eosinophils in allergic individuals demonstrate enhanced Fc α R expression, whereas neutrophils do not.²³ Mean percentages of polymorphonuclear leukocytes (PMN) and monocytes with Fc and complement receptors of asthmatic children were significantly lower than those of normals. Moreover, in allergic patients, the numbers of PMN with Fc as well as complement receptors were decreased significantly after incubation with allergen.²⁴ Our data also suggest defective phagocytosis and bactericidal function of neutrophils in PAR patients. Phagocytosis of antibody coated particles and immune complexes by PMN and macrophages is initiated by binding to Fc receptors. During chemotaxis, the Fc receptors of human neutrophils are localized to the cell's leading edge.²⁵ An abnormal regulation or expression of Fc receptors and complement receptors can partly explain the functional impairment of neutrophils in our patients.

Myeloperoxidase (MPO) is an enzyme contained in the azurophilic granules of neutrophils, which plays a major role in bacterial killing. Wang *et al.* have demonstrated that MPO is not locally elaborated in measurable quantities during suspected seasonal allergic reactions or during acute allergen challenges. In addition, they did not find any significant increase of the MPO concentration in patients with PAR.²⁶ Xue *et al.* revealed that MPO and acid phosphatase (ACP) activities in neutrophils from patients with atopic disease were significantly decreased. The results indicate that a primary deficiency of MPO and

ACP in the azurophilic granules of neutrophil exists in atopy. It was also postulated that MPO and ACP deficiency led to the reduction of the bactericidal power of neutrophils, as well as the delay of bactericidal action.²⁷ Finally, Pytsky and Filatov have found that blood cultivation of sensitized people and animals with specific allergen caused the phenomenon of specific inhibition stimulated luminol-dependent chemiluminescence of leukocytes (PhSISCL). They also assume that PhSISCL is connected with the inhibition of MPO activity.²⁸ These data lead us to assume that our experimental observations of defective phagocytosis and bactericidal function of neutrophils in PAR patients could be the result of many factors, such as decreased Fc complement receptors and a deficiency of MPO and ACP.

In conclusion, our study demonstrates that patients suffering from PAR who were in a quiescent (symptomless) interval had defective neutrophil function. The observed host defense disorders provide additional information that helps us to explain the increased susceptibility of these patients to bacterial infections. Further studies will be needed to elucidate the role of neutrophils in the pathophysiological process of PAR.

REFERENCES

- deShazo RD. Allergic rhinitis. In: Bennett JC, Plum F, Gordon NJ, Kokko JP, Ocner RK, Smith TW (eds). *Cecil Text Book of Medicine*, 20th edn. Philadelphia: WB Saunders, 1996; 1413–17.
- Bachert C, Wagenmann M, Holtappels G. Cytokines and adhesion molecules in allergic rhinitis. *Am. J. Rhinol.* 1998; **12**: 3–8.
- Papadaki HA, Xylouri I, Coulocheri S, Kalmanti M, Kafatos A, Eliopoulos GD. Prevalence of chronic idiopathic neutropenia of adults among an apparently healthy population living on the island of Crete. *Ann. Hematol.* 1999; **78**: 293–7.
- Bachert C, Van Kempen M, Van Cauwenberge P. Regulation of proinflammatory cytokines in seasonal allergic rhinitis. *Int. Arch. Allergy Immunol.* 1999; **118**: 375–9.
- Settipane RA. Complications of allergic rhinitis. *Allergy Asthma Proc.* 1999; **20**: 209–13.
- Austen FK. Diseases of immediate type hypersensitivity. In: Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL (eds). *Harrison's Principles of Internal Medicine*, 14th edn. New York: McGraw-Hill, 1998; 1867–9.
- Styrt B, Rocklin RE, Klempner MS. Characterization of the neutrophil respiratory burst in atopy. *J. Allergy Clin. Immunol.* 1988; **81**: 20–6.
- Boyum A. Isolation of lymphocytes, granulocytes and macrophages. *Scand. J. Immunol.* 1976; **5** (Suppl. 5): 9–15.
- McGregor R, Spagnuolo P, Lentek A. Inhibition of granulocyte adherence by ethanol, prednisone and aspirin measured with an assay system. *N. Engl. J. Med.* 1974; **291**: 642–5.
- Bassaris HP, Lianou PE, Dontas AS, Legakis NJ, Papavassiliou JT. Enhanced chemotaxis to gentamicin resistant strains of *Pseudomonas aeruginosa*. *J. Infect. Dis.* 1980; **141**: 507–9.
- Smith DL, Rommel F. A rapid micro method for the simultaneous determination of phagocytic–microbiocidal activity of human peripheral blood leukocytes *in vitro*. *J. Immunol. Methods* 1977; **17**: 241–7.
- Lukacs NW, Hogaboam CM, Kunkel SL *et al.* Mast cells produce ENA-78, which can function as a potent neutrophil chemoattractant during allergic airway inflammation. *J. Leukoc. Biol.* 1998; **63**: 746–51.
- Brunner T, Dahinded CA. Regulation of basophil effector functions by cytokines. *Allergy Clin. Immunol. News* 1993; **5**: 175–80.
- Bachert C, Wagenman M, Hauser U. Proinflammatory cytokines. Measurement in nasal secretion and induction of adhesion receptor expression. *Int. Arch. Allergy Immunol.* 1995; **107**: 106–8.
- Ciprandi G, Pronzato C, Ricca V, Passalacqua G, Bagnasco M, Canonica GW. Allergen-specific challenge induces intercellular adhesion molecule-1 (ICAM-1/CD54) on nasal epithelial cells in allergic subjects: Relationship with early and late inflammatory phenomena. *Am. J. Respir. Crit. Care Med.* 1994; **150**: 1653–9.
- Frew AJ, St-Pierre J, Teran LM *et al.* Cellular and mediator responses twenty-four hours after local endobronchial allergen challenge of asthmatic airways. *J. Allergy Clin. Immunol.* 1996; **98**: 133–43.
- Kumar V, Cotran R, Robbins S. *Basic Pathology*, 6th edn. Philadelphia: W.B. Saunders, 1996.
- Nagakura T, Onda T, Iikura Y, Masaki T, Nagakura H, Endo T. High molecular weight-neutrophil chemotactic activity in nasal allergy. *Allergy Proc.* 1989; **10**: 233–5.
- Tedeschi A, Palumbo G, Milazzo N, Miadonna A. Nasal neutrophilia and eosinophilia induced by challenge with platelet activating factor. *Allergy Clin. Immunol.* 1994; **93**: 526–33.
- Ternowitz T, Herlin T. Defective monocyte and polymorphonuclear leukocyte chemotaxis and clinical characteristics in atopic dermatitis. *Arch. Dermatol. Res.* 1986; **278**: 454–9.
- Hakansson L, Rak S, Dahl R, Venge P. The formation of eosinophil and neutrophil chemotactic activity during pollen season and after allergen challenge. *J. Allergy Clin. Immunol.* 1987; **83**: 933–9.
- Ivanova IP, Tananko EM, Shirinskii VS. Changes in functional activity of blood neutrophils in pollenosis patients: Effects of specific immunotherapy *Ter. Arkh.* 1999; **71**: 57–60 (in Russian).
- Monteiro RC, Hostoffer RW, Cooper MD, Bonner JR, Gartland GL, Kubagawa HJ. Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. *Clin. Invest.* 1993; **92**: 1681–5.

- 24 Hsieh K-H. Decreased Fc complement receptors on the phagocytes of asthmatic children. *Ann. Allergy* 1980; **44**: 312–16.
- 25 Walter RJ, Berlin RD, Oliver JM. Asymmetric Fc receptor distribution on human PMN oriented in a chemotactic gradient. *Nature* 1980; **286**: 724–5.
- 26 Wang D, Smitz J, Derde M-P, Clement P. Concentrations of myeloperoxidase in nasal secretions of atopic patients after nasal allergen challenge and during natural allergen exposure. *Int. Arch. Allergy Immunol.* 1996; **110**: 85–90.
- 27 Xue ZY, Lu XQ, Yu ML. Study on neutrophil enzymes in atopic disease. *Chin. Med. J. Engl.* 1993; **106**: 703–6.
- 28 Pytsky VI, Filatov OY. The mechanism of specific inhibition of stimulated chemiluminescence of polymorphonuclear blood leukocytes in allergic processes. *J. Invest. Allergol. Clin. Immunol.* 1996; **6**: 320–3.