

Neutrophil chemotactic activity (NCA) in nasal secretions from atopic and nonatopic subjects

Effect of antigen challenge

Kowalski ML, Grzegorzczak J, Śliwiska-Kowalska M, Wojciechowska B, Rożniecka M, Rożniecki J. Neutrophil chemotactic activity (NCA) in nasal secretions from atopic and nonatopic subjects. Effect of antigen challenge.

Allergy 1993; 48: 409–414. © Munksgaard 1993.

In order to elucidate the mechanism responsible for infiltration of nasal mucosa by granulocytes, we tested neutrophil chemotactic activity (NCA) in nasal lavages, by the modified Boyden chamber method, in 16 patients with perennial allergic rhinitis (AR), six ASA-sensitive patients with chronic rhinosinusitis (CRS), and seven normal, nonatopic control subjects (NC). Nasal secretions from all three groups showed significant NCA (mean 157.1 ± 54.0 , 62.2 ± 20.7 , and $39.4 \pm 11.4\%$ of FMLP chemotactic activity for AR, CRS, and NC subjects, respectively). Nasal secretions from patients with AR expressed significantly higher NCA ($P < 0.02$) than did secretions from NA patients.

NCA was unchanged by heating at 56°C for 60 min and was not susceptible to degradation by trypsin. Nasal challenge with *Dermatophagoides pteronyssinus* antigen induced clinical symptoms and resulted in significant increases in total protein and albumin concentrations in nasal lavages in AR patients, but failed to change the mean NCA activity for up to 40 min after the challenge. These results indicate that nasal secretions from both atopic and nonatopic patients express NCA, but its relation to allergic inflammation remains to be established.

M. L. Kowalski, J. Grzegorzczak,
M. Śliwiska-Kowalska,
B. Wojciechowska, M. Rożniecka,
J. Rożniecki

Department of Pulmonology and Allergology,
Faculty of Medicine, Medical Academy, Łódź,
Poland

Key words: allergic inflammation; antigenic challenge; nasal secretions; neutrophil chemotactic activity.

Dr Marek L. Kowalski
Department of Pulmonology and Allergology
Medical Academy
22 Kopcińskiego Street
90–153 Łódź
Poland

Accepted for publication 29 September 1992

The pathophysiology of chronic allergic rhinitis includes mucous gland secretion, vascular permeability leading to edema, and marked cellular infiltration of nasal mucosa (22, 23). Although eosinophils and mast cells are prominent during allergic inflammation (15, 22), the influx of neutrophils into the nasal secretions in the course of allergic reaction was also documented (2, 21). Infiltration of nasal mucosa by neutrophils may occur in nonallergic rhinitis, and these cells have enzymatic and mediator content that may contribute to chronic mucosal inflammation accompanying chronic rhinosinusitis (6). The mechanism of cellular infiltration of the airway mucosa is poorly understood, although several chemotactic factors have already been described in serum and in bronchoalveolar lavages following bronchial antigen challenge (1, 7, 19, 26). Increased generation of neutrophil chemotactic activity (NCA) in nonallergic reactions in the airways has also been reported (9, 13). Although the biochemical and cellular com-

position of nasal secretions does not directly reflect the situation in the nasal mucosa, nasal washes are a convenient source of cells and mediators presumably participating in reactions occurring in the nasal mucosa. The goal of our study was to determine the presence of NCA in nasal secretions from patients with allergic and nonallergic nasal inflammation, as compared with healthy subjects. Furthermore, we tried to assess the effect of specific nasal challenge with antigen on NCA in the nasal secretions of atopic patients.

Material and methods

Subjects

Sixteen atopic patients with perennial allergic rhinitis (AR; mean age 34.3 years), six aspirin-sensitive patients with chronic rhinosinusitis (CRS; mean age 37.9 years), and seven healthy nonatopic control volunteers (NC; 29.0 years) agreed to enter the study.

The AR patients had a history of perennial rhinitis and positive skin prick test to the house-dust mite (*Dermatophagoides pteronyssinus*) antigen. The CRS patients showed clinical and radiologic evidence of sinus disease and a history of ASA sensitivity, as confirmed by oral challenge with acetylsalicylic acid (11). Nasal steroids and ketotifen were withheld for at least 3 weeks before the study and oral antihistamines for at least 4 d. The healthy subjects had no history of allergy or nasal symptoms and had negative skin tests to a battery of inhalant allergens.

Nasal washes and challenges

Nasal lavages were performed according to the method previously described (12, 24). In brief, patients were seated in an upright position, and a soft rubber catheter was atraumatically inserted along the floor of the nasal cavity and secured to the cheek. A hand-held nebulizer was used to deliver nasal lavages with normal saline (4 ml each). Lavages were collected with a suction device. The recovery of nasal fluid ranged from 70 to 90%, depending on the severity of nasal symptoms. The lavages were kept on ice and subsequently frozen.

Nasal challenges were performed with a nasal pump spray delivering 120 μ l per puff. Only one nasal cavity was challenged with buffer; and then in 10-min intervals with two concentrations of *D. pteronyssinus* allergen extract (Beecham-Bencard, Brentford, UK): 1:100 and 1:10 dilution of the original bronchial provocation solution. Before the challenge, a saline washing was collected to serve as base line. After each challenge, lavages were collected for 10 min and then every 10 min up to 30 min after the 1:10 concentration of the allergen. Clinical symptoms were recorded by the symptom score technique, grading the responses as none = 0, mild = 1, moderate = 2, or severe = 3. A composite nasal score was calculated as the sum of individual scores for blockage, sneezing, and secretion.

Biochemical determinations

Total protein concentration in the nasal lavages was determined by the method of Lowry et al. (14), albumin was measured by rocket immunoelectrophoresis, and lysozyme was measured by the lyso-plate assay method (20).

Neutrophil chemotactic assay

Neutrophils were purified from heparinized human blood by the method of Boyum (3). In brief, 20 ml venous heparinized blood was mixed with the same volume of PBS and then underlayered with 10 ml Histopaque (Sigma Co., St Louis, MO, USA) and

centrifuged at $400 \times g$ for 20 min. The bottom layer containing the erythrocytes and neutrophils was suspended in a similar volume of 3% dextran (Sigma, St Louis, MO, USA) in normal saline for 20 min. The neutrophil-rich layer was aspirated, resuspended in red-cell lysing buffer, and then washed twice with HBSS. A cell count was performed, and viability was determined by trypan blue dye exclusion. Cells were then resuspended in HBSS containing 0.5% w/v ovalbumin as 1×10^{-6} PMNs/ml. The cell population included 95–97% neutrophils, 1–2% eosinophils, and 3–4% mononuclear cells.

The chemotactic activity present in nasal washes or in gel chromatography fractions was measured in a 48-well chemotaxis chamber (Neuro Probe, Bethesda, MD, USA) (5). A 3- μ m pore, 10- μ m-thick, polyvinyl pyrrolidone-free polycarbonate filter (Nucleopore Pleasanton, CA, USA) was used (8). Twenty-five microliters of lavage fluid was placed in the bottom wells of the chamber. The control wells contained buffer or FMLP in a concentration of 10^{-7} mol/l. Fifty microliters of the cell suspension was introduced into each well of the top plate, and the chamber was incubated at 37°C for 90 min. The membrane was fixed in absolute methanol for 5 min and stained with Wright's stain. The NCA was measured by counting the number of granulocytes that traversed the membrane in eight random high-power microscopic fields. Data were expressed as numbers of cells migrating per 8 HPF or as a percentage of the chemotaxis induced by 10^{-7} FMLP. All procedures were run in a blind fashion, and each sample was assayed in duplicate. The intraday variation of the method was 15.1% (CV) and the interday variation 19.8% (CV).

Statistical analysis

Results are presented as mean $\pm$ SEM, and significance was calculated by a one-tailed Wilcoxon rank sum test or Student's paired t-test. Correlations between measurements were determined by Spearman's rank correlation test.

Results

Proteins and NCA in basal nasal washes

The base-line nasal lavages of all three group studied are compared in Table 1. Although AR and CRS patients tended to have higher concentrations of total protein and albumin than did NC, the mean differences were not statistically significant. The albumin percentage was significantly higher in both AR and CRS patients than in NC.

Preliminary experiments demonstrated that undiluted nasal washes from atopic patients contained a significant amount of NCA, and the effect of dilution

Table 1. Proteins and neutrophil chemotactic activity (NCA) in basal nasal washes

	Atopic rhinitis	ASA-sensitive CRS	Normal controls
Number of subjects	16	6	7
Total protein ($\mu\text{g/ml}$)	211.8 ± 51	185.2 ± 45.8	149.0 ± 38.1
Albumin ($\mu\text{g/ml}$)	67.4 ± 17.8	35.3 ± 11.1	18.5 ± 7.5
Albumin %	$22.5 \pm 4.2^*$	$20.4 \pm 4.0^*$	10.6 ± 2.7
Lysozyme ($\mu\text{g/ml}$)	18.1 ± 4.1	ND	12.4 ± 2.8
NCA (% of 10^{-7} FMLP-induced chemotaxis)	$157.3 \pm 54.8^{**}$	62.2 ± 20.7	39.4 ± 11.4

Values are means $\pm$ SEM.

* $P < 0.05$, as compared with normal controls (Wilcoxon rank sum test).

** $P < 0.02$, as compared with normal controls.

ND=not done.

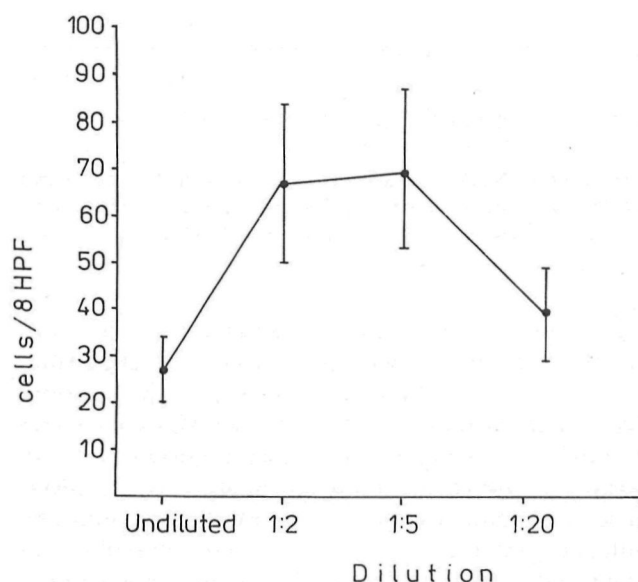


Fig. 1. Effect of dilution of nasal washes from 10 atopic patients on NCA (mean $\pm$ SEM).

on this activity was assessed. As shown in Fig. 1, dilution of the nasal lavage samples increased NCA, and the greatest chemotactic activity was present in samples diluted 1:2 and 1:5 in saline; further dilution again decreased NCA. Therefore, in all experiments the nasal washes were diluted 1:5 before assaying NCA.

Basal nasal washes obtained from all three groups contained NCA that was significantly higher than the buffer ($P < 0.001$; $P < 0.05$ and $P < 0.001$ for AR, CRS, and NC patients, respectively; Wilcoxon rank sum test). NCA in nasal lavages of AR, but not CRS patients, was significantly higher than in NC subjects (Table 1).

Correlation between concentration of proteins and NCA

In atopic patients, NCA in nasal washes significantly correlated with the concentration of total protein ($r = 0.55$; $P < 0.02$), albumin ($r = 0.64$; $P < 0.01$), al-

bumin percentage ($r = 0.70$; $P < 0.001$), and lysozyme activity ($r = 0.49$; $P < 0.04$; Spearman's rank correlation coefficient).

Partial characterization of NCA

When an aliquot of the nasal lavage was trypsinized and NCA was compared with that of native material, no significant difference between those two samples was observed (migration toward native material 41.7 ± 7.9 cells/8 HPF; migration towards trypsinized lavages 48.5 ± 8.1 cells/8 HPF; $P > 0.05$). Heating of the nasal washes at 56°C for 60 min did not diminish NCA (NCA was 103.2 ± 20.2 cells/8 HPF and 107.0 ± 21.6 for unheated and heated samples, respectively).

NCA in nasal secretion induced by antigen challenge

Allergen challenge resulted in a gradual increase in symptoms in 8 of 16 patients. Accordingly, patients were divided into two groups: group P (positive response; eight patients) with clinical symptoms after the challenge and group N (no response; eight patients) without clinical response to the antigen. We observed only in group P a significant increase in the concentration of total protein, albumin, and albumin percentage (data not shown) in nasal washes collected after insufflation of the highest concentration of antigen (Fig. 2a, b, and c).

Fig. 3 shows that after nasal antigen challenge there was no significant change in the mean NCA in lavages collected for up to 30 min after delivery of the highest concentration of the antigen (i.e. up to 40 min after the first antigen dose). Nor was there a significant difference between mean NCA in groups P and N at any of the time points studied. Analysis of maximal changes in NCA for individual patients, without regard to the time point after the challenge, revealed that in three patients from group P and four patients from group N, increases in NCA occurred (mean increase $+127.8\%$ and $+155.1\%$ for group P and group N, respectively).

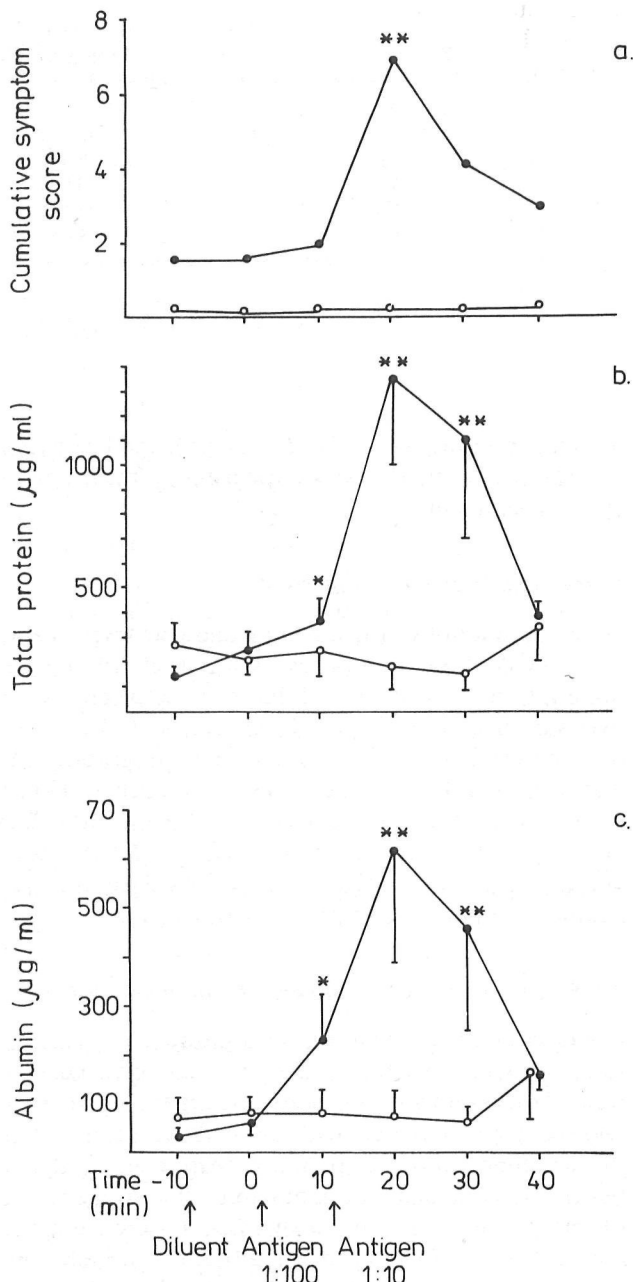


Fig. 2. Mean cumulative symptom score (a), total protein (b), and albumin concentration (c) in nasal lavages after antigen challenge. Open circles represent nonresponders ($n = 8$); closed circles, patients with clinical symptoms after antigen challenge ($n = 8$). * $P < 0.05$; ** $P < 0.01$, as compared with base line (paired samples t-test).

Discussion

Our study demonstrated for the first time that basal nasal secretions of both healthy persons and patients with chronic rhinitis (allergic or ASA-sensitive) contain significant chemotactic activity to neutrophils. The presence of this activity in asymptomatic subjects is consistent with observations that nasal

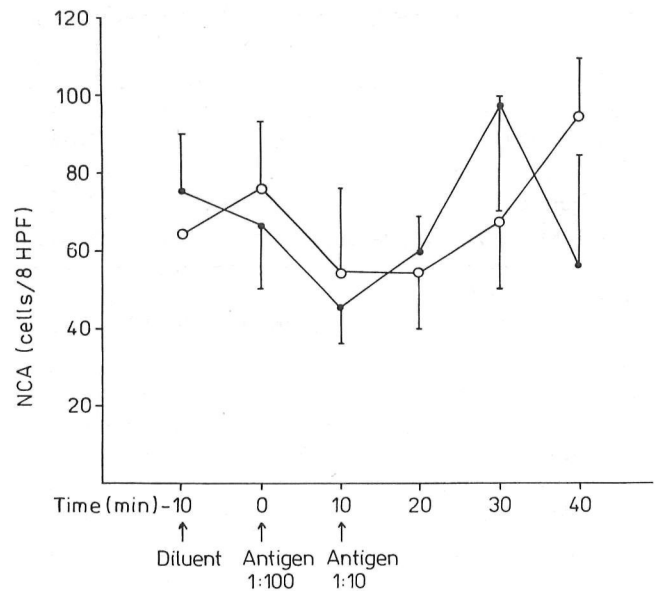


Fig. 3. Mean NCA in nasal lavages after antigen challenge. Open circles represent nonresponders; closed circles represent patients with clinical and biochemical reactions after antigen challenge.

secretions even in healthy persons contain a significant (as compared with patients with allergic rhinitis) number of PMN leukocytes (17). It has been hypothesized that the presence of PMN leukocytes in healthy persons may be an expression of physiologic inflammation of the nasal mucosa, resulting from continuous exposure to microorganisms and inhaled particles (17). Basal secretions obtained from patients with allergic rhinitis expressed significantly higher NCA than did secretions of control healthy persons, and the NCA correlated with local inflammation, as reflected by concentrations of both vascular protein (albumin) and glandular protein (lysozyme). The observation that after antigen challenges, despite increases of several hundreds of percent in protein concentration, NCA did not increase in parallel, suggested that the activity was not directly related to any of these proteins but rather to the intensity of the inflammatory process in the nasal mucosa. Accordingly, we have observed that by diluting nasal secretions, one can achieve the enhancement of chemotactic activity. This could result from a loss of some inhibitors of chemotaxis in nasal secretions. The presence of various naturally occurring inhibitors of chemotaxis that can mask chemotactic effect has been described in the whole sera (25). It is likely that similar combinations of multiple, naturally occurring chemotactic factors and inhibitors are present in the nasal secretions.

We attempted to define the biochemical nature of NCA from crude nasal washes, confirming its heat stability and resistance to enzymatic degradation by proteolytic enzyme. Although heat stability is char-

acteristic of the HMW-NCF, its resistance to proteolytic degradation is not (10). Further work is needed to determine the nature and molecular character of NCA detected in nasal washes.

Only half of house-dust-mite-sensitive patients demonstrated a positive clinical response to nasal allergen challenge, reflecting previously described variability in the response of atopic subjects to nasal allergen challenge (4). In parallel with clinical response, these patients had significant and very consistent increases in biochemical parameters of mucosal allergic reaction: total protein, albumin, albumin percentage, and lysozyme. The present study shows that antigen-induced immediate allergic reaction is not consistently associated with an increase in NCA in nasal lavages. The mean increase of NCA 30 min after antigen challenge in patients with apparent nasal allergic reaction was only 28% above basal values and was comparable with the mean NCA observed 40 min after antigen challenge in the nonresponsive group. When individual data were analyzed, some patients from both groups (responders and nonresponders) demonstrated noticeable changes from base-line NCA on a single occasion, 10–40 min after the challenge. However, the changes in NCA were not related to the allergic response, since the magnitude of these changes was similar both in patients who experienced clinical and biochemical responses to antigen and in patients without any symptoms after the challenge; hence, these changes could not be unequivocally attributed to allergic reaction.

Our results contrast with those of previous studies in which antigen challenges resulted in apparent increases in NCA. Nagakura et al. (18) studied three children with allergic rhinitis and demonstrated that local instillation of antigen was associated with an increase in NCA, 10 min after the challenge. However, in that study the reaction was assessed only by clinical symptoms, and no control challenges or time course of NCA generation was reported. On the other hand, Mackay et al. (16) reported release of the HMW-NCA in time-dependent fashion after nasal allergen challenge. Unfortunately, their data are available only in abstract form, and that does not allow more detailed comparison with our study. It is possible that the discrepancies between our study and the others may result from method of nasal lavage, e.g. different volume of washes or processing of samples. Since we have shown that by diluting the secretions one may influence chemotactic activity, it may be hypothesized that during the challenge the relation between NCA and possible inhibitory factors might have changed, thus resulting in the observed variability.

In conclusion, our results indicate that NCA is present in nasal secretions of both atopic and non-

atopic subjects. However, further investigations are required in order to characterize its origin and also its relevance to nasal mucosal inflammation.

Acknowledgment

We thank Mrs B. Lipowska for technical assistance.

References

1. ATKINS PC, NORMAN M, WEINER H, ZWEIMAN B. Release of neutrophil chemotactic activity during immediate hypersensitivity reactions in humans. *Ann Intern Med* 1977; 86: 415–18.
2. BASCOM RM, PIPKORN U, LICHTENSTEIN LM, NACLERIO RM. The influx of inflammatory cells into nasal washings during the late response to the antigen challenge: effect of systemic steroid pretreatment. *Am Rev Respir Dis* 1988; 138: 406–12.
3. BOYUM A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation of 1 g. *Scand J Lab Invest* 1968; 21 (Suppl. 97): 77–89.
4. CASTELLS M, SCHWARTZ LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. *J Allergy Clin Immunol* 1988; 82: 348–55.
5. FALK W, GOODWIN RH Jr, LEONARD EJ. A 48-well microchemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J Immunol Methods* 1980; 33: 239–47.
6. FRYKSMARK U, JANNERT M, OHLSSON K, TEGNER H. Antileukoprotease in patients with maxillary sinusitis. *Rhinology* 1985; 23: 247–51.
7. GOSSET P, TONNEL AB, JOSEPH M, et al. Secretion of chemotactic factor for neutrophils and eosinophils by alveolar macrophages from asthmatic patients. *J Allergy Clin Immunol* 1984; 74: 827–34.
8. HARVATH L, FALK W, LEONARD E. Rapid quantitation of neutrophil chemotaxis: use of polyvinylpyrrolidone-free polycarbonate membrane in a multiwell assembly. *J Immunol Methods* 1980; 37: 39–45.
9. HOLLINGSWORTH HM, DOWNING ET, BRAMAN SS, GLASSROTH J, BINDER R, CENTER DM. Identification and characterization of neutrophil chemotactic activity in aspirin-induced asthma. *Am Rev Respir Dis* 1984; 130: 373–9.
10. KAY AB, LEE TH. Neutrophil chemotactic factor of anaphylaxis. *J Allergy Clin Immunol* 1982; 70: 317–20.
11. KOWALSKI ML, GRZELEWSKA-RZYMOWSKA I, SZMIDT M, ROZNIECKI J. Bronchial hyperreactivity to histamine in aspirin sensitive asthmatics: relationship to aspirin threshold and effect of aspirin desensitization. *Thorax* 1985; 40: 598–602.
12. KOWALSKI ML, SLIWINSKA-KOWALSKA M, IGARASHI Y, et al. Nasal secretions in response to aspirin. *J Allergy Clin Immunol* (in press).
13. LEE TH, NAGAKURA T, PAPAGERGIU N, IKIRA Y, KAY AB. Exercise-induced late asthmatic reaction with neutrophil chemotactic activity. *N Engl J Med* 1983; 308: 1502–5.
14. LOWRY OL, ROSEBROUGH NJ, FARR AL, RANDAL RJ. Protein measurement with the folin reagent. *J Biol Chem* 1951; 193: 265–75.
15. LOZEWICZ S, GOMEZ E, CLAGUE J, GATLAND D, DAVIES RJ. Allergen-induced changes in the nasal mucous membrane in seasonal rhinitis: effect of nedocromil sodium. *J Allergy Clin Immunol* 1990; 85: 125–31.
16. MACKAY JA, CROMWELL O, KAY AB. Allergen-induced

- release of high molecular weight neutrophil chemotactic activity in nasal secretions from patients with allergic rhinitis (Abstract). *J Allergy Clin Immunol* 1986; 77: 183.
17. MYGIND N. Nasal allergy. 2nd ed. London: Blackwell Scientific, 1979: 3-38.
18. NAGAKURA T, ONDA T, IKURA Y, et al. *In vitro* and *in vivo* antigen-induced release of high-molecular weight neutrophil chemotactic activity from human nasal tissue. *Am J Rhinology* 1988; 2: 7-11.
19. NAGY L, LEE TH, KAY AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. *N Engl J Med* 1982; 306: 497-501.
20. OSSERMAN EF, LAWLOR DP. Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia. *J Exp Med* 1966; 124: 921-51.
21. PELIKAN Z, PELIKAN-FILIPEK M. Cytologic changes in nasal secretions during immediate nasal response. *J Allergy Clin Immunol* 1988; 82: 1103-12.
22. PIPKORN U, KARLSSON G, ENERBACK L. The cellular response of the human allergic mucosa to natural allergen exposure. *J Allergy Clin Immunol* 1988; 82: 1046-54.
23. RAPHAEL GD, BARANIUK JN, KALINER MA. How and why the nose runs. *J Allergy Clin Immunol* 1991; 87: 457-67.
24. RAPHAEL GD, DRUCE HM, BARANIUK JN, KALINER MA. Pathophysiology of rhinitis. I. Assessment of sources of protein in methacholine-induced nasal secretions. *Am Rev Respir Dis* 1988; 138: 413-20.
25. TILL G, WARD PA. Two distinct chemotactic factor inactivators in human serum. *J Immunol* 1975; 114: 843-47.
26. VENGE P, DAHL R, HAKANSON L. Heat-labile neutrophil chemotactic activity in subjects with asthma and after allergen inhalation: relation to the late asthmatic reaction and effects of asthma medication. *J Allergy Clin Immunol* 1987; 80: 679-88.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.