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Study of nasal cytology in atopic patients after nasal allergen challenge*

Deyun Wang[1], Peter Clement[1], Marc De Waele[2], Marie-Paule Derde[3]

[1] Department of Otorhinolaryngology, University Hospital, Free University, Brussels, Belgium
[2] Department of Haematology, University Hospital, Free University, Brussels, Belgium
[3] Department of Biostatistics, University Hospital, Free University Brussels, Belgium

SUMMARY

In order to study the normal and pathological inflammatory cell population in nasal secretions, nasal microsuction has been performed in 18 atopic patients and 10 healthy volunteers after nasal allergen and/or PBS challenge. After cytospin, the samples have been stained with May-Grünwald-Giemsa solution. Three hundred inflammatory cells have been counted by light microscopy, and the percentage of each cell type has been calculated. Results show that only a significant increase ($p < 0.01$) in the percentage of eosinophils 1-10 h after nasal allergen challenge occurs. In general, this finding correlates well with the symptom of nasal obstruction as measured by passive anterior rhinomanometry (PAR) during the late phase, but not with the number of sneezes. In agreement with the literature, late-phase nasal obstruction is shown to be accompanied by an increase in the percentage of eosinophils in nasal secretions. The potential role of eosinophils in the pathogenesis of the late phase in the inflammatory events after nasal allergen challenge has again been confirmed by our study. This study further confirms the usefulness of nasal microsuction as a sampling technique, providing uniform and adequate nasal cytological specimen for the analysis of nasal cytology.

Key words: cytology, nasal secretions, allergic rhinitis, nasal challenge

INTRODUCTION

Many studies have reported that infiltration of the nasal mucosa by various inflammatory cells plays an important role in nasal allergic inflammation. Therefore, appropriate nasal cytological investigation offers interesting perspectives into the pathophysiological mechanism of allergic rhinitis.

Until now, different sampling techniques have been used, such as nasal biopsy (Bentley et al., 1992), nasal scrapings (Dolovich et al., 1989; Okuda et al., 1991), nasal smears (Malmberg and Holopainen, 1979; Bogaerts and Clement, 1981), nasal lavage (Wachs et al., 1989), and nasal blowing (Pelikan and Pelikan-Filipek, 1989; Okuda et al., 1991). However, the advantages and disadvantages of these sampling techniques have been discussed as a matter of controversy and conjecture for many years. Recently, the authors have used the microsuction technique (Biewenga et al., 1991) for collecting nasal secretions after nasal allergen challenge. The collected cells are counted by using a simple light-microscopical technique. The aim of this study has been to determine the cell population of nasal secretions. The second objective has been to understand at what percentage the inflammatory cells in nasal secretions are to be considered as pathological during the allergic reaction.

MATERIAL AND METHODS

Patients

Eighteen atopic patients (11 males and seven females, aged from 18 to 45 years; mean age: 28 years) were enrolled in this study. Grass pollen allergy was confirmed by medical history, skin test and nasal grass pollen challenge. A control group consisted of 10 healthy volunteers (five males and five females, aged from 20 to 43 years; mean age: 28 years). All of them had normal findings upon rhinoscopy, routine blood chemistry parameters, and haematological examinations. The study was performed three months after the pollen season. The subjects gave their informed consent. This study was approved by the Local Ethics Committee.

Nasal aspiration

The authors used Biewenga's aspiration system. The samples were collected by repeated aspiration from the middle meatus

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and from the floor of both nasal cavities, into a pre-weighed plastic sampling tube. This was immediately followed by aspiration of a known aliquot (1.0 ml) of phosphate-buffered saline (PBS, pH 7.4) including 10% Mesna (Mistabron, UCB Pharmaceutics company, Belgium). Mesna acts by disrupting the disulfide bonds of the mucus polypeptide chains, which is especially necessary to separate the gel phase from the sol phase.

Nasal challenge and sampling procedure

Nasal challenge was carried out by nasal aerosol application with a Heyer nebulizer (Heyer Company, Germany; Clement et al., 1981) of the control solution (PBS, pH 7.4) and grass pollen mixtures (20,000 AU/ml; HALAB Allergy Service, Belgium). The temperature of the aerosol was maintained at 28–30°C.

Initially, two consecutive samples were collected in the early morning (about 8:30 a.m.) as baseline controls. Fifteen minutes later, nasal challenges with PBS and allergen (PBS again for the control group) were performed at 15-min intervals. Nasal secretions were then collected immediately after each challenge and every hour for 10 h, as well as 24, 32, 48, and 56 h after the initial nasal allergen (or PBS in the control group) challenge. A total of 18 samples were obtained from each subject during these three days. The samples were cooled on ice immediately after sampling, then mixed in a Vortex mixer, centrifuged at 1,000*g* at 4°C for 15 min. The supernatants were stored at -20°C or -80°C for subsequent determination of the mediators.

Cellular examination

After removing the supernatant, the pellets were resuspended in 1 ml 5% albumin and 0.1 ml Mesna and incubated at 37°C for 30 min, or longer when there remained much sticky mucus in the collected secretions. After cytospin, the samples were stained with May-Grünwald-Giemsa solution. Three hundred inflammatory cells were counted by light microscopy, and the percentage of each cell type was then calculated.

Objective symptoms

The number of sneezes was recorded in absolute number. Nasal airway resistance (NAR) was measured by passive anterior rhinomanometry (PAR), and expressed in decaPa/cm^3/s of the left and right sides. A 100% increase of NAR at one or both nasal cavities was considered a nasal obstruction.

Statistical evaluation

The Wilcoxon rank sum test was used to compare the values at each of the sampling times with the baseline value after nasal PBS and/or allergen challenge. Only a p-value of <0.01 was considered statistically significant in order to correct multiple comparison.

RESULTS

After nasal PBS challenge, there occurred no increase in either the number of sneezes or in the NAR of the subjects in the control group. In the patient group, only one patient failed to show any response after nasal allergen challenge, and he was therefore disregarded for further evaluation. Sneezes were counted in 13 patients (76%) during the early phase (range: 4–20 times) and only in six patients (29%) during the late phase (4 h later; range: 1–5 times). Seventeen out of 18 patients (94%) showed unilateral or bilateral nasal obstructions immediately after nasal allergen challenge. Fourteen out of 17 patients (82%) showed a late nasal obstruction appearing at one or both sides. Following these findings, the authors selected the nasal side with a higher value of the NAR and calculated their median in order to better understand the real variation by changing nasal resistance after nasal allergen challenge (Figure 1).

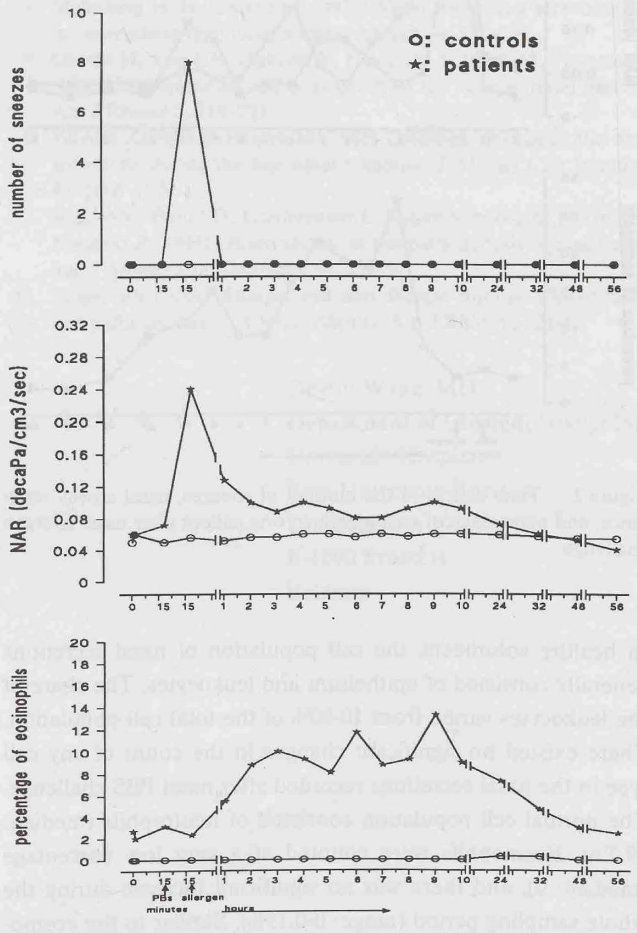

Figure 1. Time course of the median values of the number of sneezes, nasal airway resistance, and percentage of eosinophils in atopic patients (n=17) after nasal allergen challenge, and in the control group (n=10) after nasal PBS challenge.

A total of 504 cytospins were obtained from all patients and healthy volunteers. Of all cytospins, approximately 4% was of poor quality and/or contained insufficient numbers (<300 cells) of inflammatory cells, and these were excluded from further evaluation. Red blood cells were rarely seen, except in those cases where damage of the mucous membrane had occurred, resulting in a poor cytospin. Microscopical examination showed that there existed no significant differences in cell population between the first two consecutive samples.

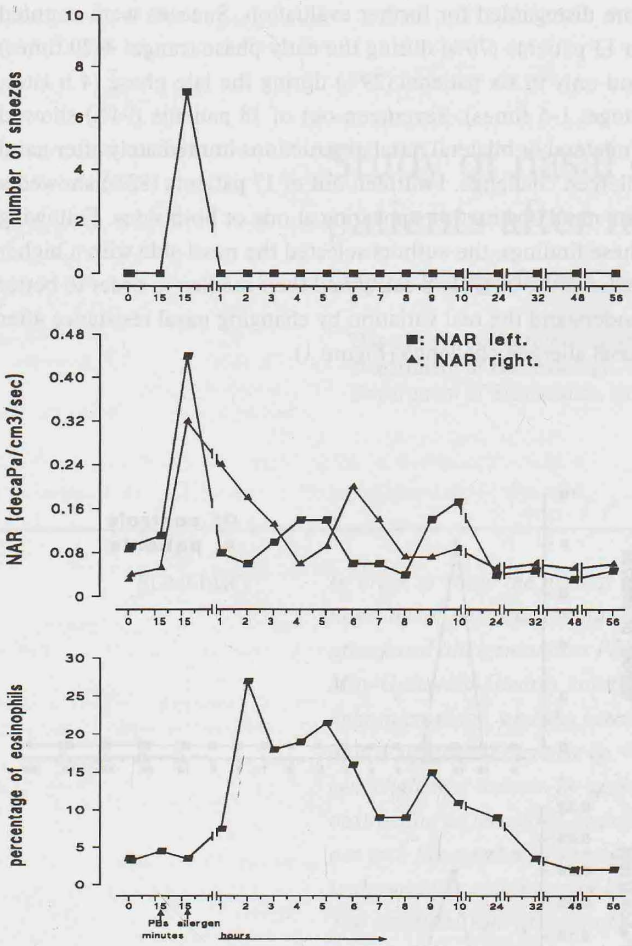

Figure 2. Time course of the number of sneezes, nasal airway resistance, and percentage of eosinophils in one patient after nasal allergen challenge.

In healthy volunteers, the cell population of nasal secretions generally consisted of epithelium and leukocytes. The share of the leukocytes varied from 10-90% of the total cell population. There existed no significant changes in the count of any cell type in the nasal secretions recorded after nasal PBS challenge. The normal cell population consisted of neutrophils (median: 99.7%). Eosinophils were counted at a very low percentage (median: 0), and there was no significant increase during the whole sampling period (range: 0-0.15%). Similar to the eosinophils, lymphocytes were counted also at a very low percentage (median: 0.3%). The variation of the percentage of lymphocytes was very slight during the whole sampling procedure (0.3-1.3%). Other cells (i.e., mast cells, basophils, and monocytes) were rarely found.

Eosinophils were the predominant cellular response after allergen challenge in the patient group. There existed a significantly-higher median baseline percentage of eosinophils (-median: 2.7%) in the patient group than in the control group. A significant increase of eosinophils (median: 5.5%) appeared 1 h after nasal allergen challenge. It remained significantly higher for 10 h (range: 8.1-13.3%), and started to decrease 24 h later (Figures 1-2). There existed no significant change in the median percentage of lymphocytes. The median percentage of lymphocytes remained very low (range: 0.7-2.3%) in the nasal secretions. The percentage of neutrophil decrease was inversely proportional to the increase in eosinophils. The results of the other cell types remained the same in comparison to the control group.

DISCUSSION

The history of nasal cytology and allergy dates back to Bizzozera in 1887, who was the first to demonstrate the presence of eosinophils in nasal mucus. Later, Hansel stressed the importance of eosinophils as a diagnostic marker of allergy (Zeiger, 1989). So far, the principal cellular infiltrate accompanying a wide variety of inflammatory processes in the respiratory tract includes granulocytes of various lineages (neutrophils, macrophages, eosinophils, and metachromatic cells). In addition, variable changes in structural compartments (epithelial cells and fibroblasts) are also observed (Denburg et al., 1990). The authors have tried to find out at what percentages these cells in nasal secretions are to be considered pathological.

Furthermore, we wanted to know if it is possible with a simple and reliable nasal sampling technique to provide diagnostic information about allergic rhinitis or to follow-up the effect of any therapy. In this study we, therefore, have used the microsuction technique for collecting nasal secretions. One of the major advantages of this method of nasal sampling is that the cell distribution on cytospin is much more uniform. The density of cells on cytospin can easily be controlled by the investigator. The samples obtained from scrapings and smears, due to the sticky mucus resulting in clusters of cells, are only of limited use for quantitative evaluation.

The increase of eosinophils in nasal secretions has been observed to start 1 h (in 94% of the cases) after nasal allergen challenge, and this increase is statistically significant as compared to the baseline ($p < 0.01$). This increase in eosinophils seems to correlate with the symptom of nasal obstruction measured by passive anterior rhinomanometry. Infiltration of the nasal mucosa by eosinophils, especially during the late-phase nasal response after nasal allergen challenge, has been well documented by many studies. The exact mechanism of activation and infiltration of eosinophils is not well-established as yet.

Until now, evidence shows that activated eosinophils act both directly and indirectly on the nasal epithelium by releasing inflammatory mediators. As demonstrated *in vitro*, activated eosinophils release granule-associated proteins that are cytotoxic for respiratory epithelial cells (Frigas et al., 1986). Following our data, eosinophilia in nasal secretions occurs about 10 h after nasal allergen challenge. Several important questions remain unanswered, such as: what is the activation factor of the eosinophils? and when are the eosinophils activated after they have migrated into the nasal secretions?

The other cells are certainly not excluded from playing an important role in nasal allergic inflammation. The microsuction technique did not show important quantitative changes in this cell population. A cytogram of nasal secretions allows only the study of changes in the percentages of the different cell populations. A drawback of this way of assessing nasal cytology is that

it does not give absolute quantitative data because of the diversity of the different cell populations (leukocytes, mast cells, and epithelial cells). Therefore, the presence of the different cell types in nasal secretions can only be given as a percentage. The percentage of neutrophil decrease was inversely proportional to the increase in eosinophils. The other cells such as mast cells and/or basophils are only seen rarely, because they are either sited in the lamina propria or located near the epithelial cells, needing forceful scraping or brushing.

In summary, by using the nasal microsuction the authors have provided direct evidence of an important increase in the percentage of eosinophils in nasal secretions of atopic patients 1–10 h after nasal allergen challenge. This finding generally correlates well with the symptom of nasal obstruction measured by passive anterior rhinomanometry. The potential role of eosinophils in the pathogenesis of the late phase after nasal allergen challenge has been confirmed by our study. The migration and activation mechanism of eosinophils is beyond the possibility of the technique. On the other hand, the authors believe that nasal microsuction is a very useful and easy sampling technique providing uniform and adequate nasal cytological specimen for studying the pathophysiology of any disease of the nasal mucosa.

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Deyun Wang, MD
Department of Otorhinolaryngology
University Hospital
Free University Brussels
Laarbeeklaan 101
B-1090 Brussels
Belgium