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NASAL LAVAGE FLUID EXAMINATION AND RHINOMANOMETRY IN THE DIAGNOSTICS OF OCCUPATIONAL AIRWAY ALLERGY TO LABORATORY ANIMALS

ANNA KRAKOWIAK[1], URSZULA RUTA[1], PAWEŁ GÓRSKI[2], SYLWIA KOWALSKA[3] and CEZARY PAŁCZYŃSKI[1]

[1] Department of Occupational Diseases
Nofer Institute of Occupational Medicine
Łódź, Poland
[2] Division of Pneumonology and Allergology
Medical University
Łódź, Poland
[3] ENT and Occupational Audiology Unit
Nofer Institute of Occupational Medicine
Łódź, Poland

Abstract
Objectives: The purpose of this study was to compare the cellular and biochemical findings in the nasal lavage fluid (NALF) and nasal resistance changes due to a challenge with laboratory animal allergens in 25 patients with occupational asthma and rhinitis, in 22 patients with atopic asthma and rhinitis sensitized to house dust mite, and in 15 healthy subjects. **Methods:** Skin prick tests with common and occupational allergens, total serum IgE level, specific anti - allergens IgE, spirometry and nasal lavages were performed. **Results:** In patients with occupational airway allergy, nasal symptoms of varying severity developed directly after specific nasal challenge. The total symptom score immediately and 24 h after specific challenge was significantly correlated with expiratory nasal resistance (ENR). The percentage of eosinophils and basophils in NALF increased significantly 5 and 24 h after specific challenge in patients with occupational asthma and it was correlated with ENR. The authors did not observe any significant increase in the percentage of eosinophils, basophils and in the level of albumin in NALF of patients with non-occupational allergy and healthy subjects at any time-point after specific challenge. None of the healthy subjects and patients with non-occupational allergy developed either allergic symptoms or increased ENR after the challenge with laboratory animal allergens and placebo. **Conclusions:** The prolonged increase in the percentage of eosinophils, basophils and in the level of albumin seems to reflect allergic inflammation. The intensity of inflammation during the specific reaction is related to the symptom score and to ENR in occupational allergics.

Key words:
Nasal lavage, Occupational asthma, Laboratory animals

INTRODUCTION

Occupational allergy is an important health problem for those exposed to animals. Exposure to laboratory animals such as rats and mice occurs at academic and research institutions among people undertaking animal studies and those involved in animal breeding. The diagnosis of laboratory animal allergy is based on a history of nasal or chest symptoms in persons who encounter these symptoms at

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Address reprint requests to C. Pałczyński MD, PhD, Department of Occupational Diseases, Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland (e-mail: cpalczyn@imp.lodz.pl).

work. Objective evidence to support the diagnosis can be obtained from a specific immunological response (IgE), skin prick tests (SPTs) with occupational allergens, work-related changes in peak expiratory flow rate (PEFR) and inhalation tests.

In the diagnosis of occupational airway sensitization, it can be very difficult to differentiate between specific, allergic and nonspecific irritant reactions. Recently, the nasal lavage fluid (NALF) examination and rhinomanometry have become important research instruments to study cellular events and nasal resistance changes in asthmatic airways after allergen challenge [1–3].

The purpose of this study was to compare the cellular and biochemical findings in NALF and nasal resistance changes due to a challenge with laboratory animal allergens between patients with (a) occupational asthma and rhinitis (patients with occupational allergy); (b) bronchial asthma and rhinitis who were sensitized to house dust mite (patients with non–occupational allergy), and (c) non-atopic healthy subjects as controls.

MATERIALS AND METHODS

Subjects

The study group consisted of 25 patients (mean age, 41.9 ± 11.6 years) with occupational bronchial asthma and allergic rhinitis who were admitted to the Department of Occupational Diseases in 1997–1999. The diagnosis of bronchial asthma was based on the criteria of the American Thoracic Society [4], and those of occupational allergy, on a positive history and a significant (>20%) fall in PEFR induced by occupational exposure. The patients also displayed positive skin prick tests with occupational allergens of laboratory rats and mice, and/or the presence of specific IgE.

The control group consisted of 22 subjects (mean age, 45.5 ± 8.2 years) suffering from atopic asthma and rhinitis who were sensitized to house dust mite, and of 15 healthy volunteers (mean age, 42.9 ± 10.4 years).

The study was approved by the local Ethical Committee and all the subjects presented their written consent prior to the study.

Study protocol and challenge procedure

The study was designed as a two-stage, crossover, single blind trial. At the first stage, all subjects were challenged with placebo – potato flour. At least seven days later, during the second stage, the subjects were challenged with occupational allergens (rat and mouse fur and dust collected from animal cages). Each subject underwent four nasal lavages immediately before provocations and 30 min, 5 and 24 h after provocations with placebo and occupational allergens.

The bronchial challenge test was performed in an inhalation chamber. At the first stage of the study, each subject was instructed to sift potato flour (as placebo) in the amount of about 0.5 kg and then after seven days, at the second stage, to mix the materials obtained from their workplace (dander of rats and mice; dust taken from the animal cages).

The whole challenge lasted 15 min.

Study design

Each subject had a medical history collected and a physical examination and spirometry were carried out. Skin prick tests were performed using common and occupational allergens such as *Dermatophagoides ptheronyssinus*, pollens, moulds, trees, house dust, rat and mouse dander (Allergopharma, Germany). Negative control was made with an allergen diluent, and the positive one, with histamine solution. All the sites were examined 20 min post-challenge: the grading of the wheal (positive: 4 mm > control) and flare (positive: 5 mm > control) was checked up following standard methods. Total serum IgE and the presence of specific IgE (RAST) were evaluated (Pharmacia, Sweden).

Symptom score

The number of sneezes and the degree of mucosal oedema, rhinorrhea and itching were evaluated. The total symptom score (SS) ranged from 0 to 7 and represented the sum of the scores for sneezing (no sneezes – 0 points; 1–4 sneezes – 1 point; >4 sneezes – 2 points) rhinorrhea (none – 0 points; mild – 1 point; abundant – 2 points); mucosal oedema (none – 0 points; mild – 1 point; nasal

block – 2 points). Positive clinical challenge was defined as > 3 points [5].

Nasal washings processing

Before the provocation, each nostril was washed 10 times with 6 ml saline using the "nasal pool" device (5-ml syringe closely fitting the nostril). Nasal washings were collected immediately before the challenge and 30 min, 5 and 24 h after the challenge. All lavages were always performed on the same side of the nasal cavity.

The nasal washings were centrifuged (10 min at 1000 rpm) to isolate the cells pellet and the supernatant. The obtained sediment was washed with sterile phosphate-buffered saline (Dulbecco, Sigma) and 0.1% human serum albumin (HSA, Behringwerke A.G.) and then suspended in 1 ml buffer with HSA. The cells were stained following: (a) the Turk method for leukocytes; (b) the Dunger method for eosinophils; and (c) 0.06% toluidine blue in 30% ethanol for basophils (metachromatic cells). The cells were counted in the Fuchs-Rosenthal chamber. The number of cells in 1 ml of the recovered fluid was determined.

The samples were further centrifuged at 2000 rpm for 5 min, transferred onto a slide, and air-dried. The slides were stained following the Giemsa method. The first 200 cells on each slide were classified into eosinophils, basophils and neutrophils. Total protein content in the supernatant was evaluated with the Lowry method [6]. Albumin concentration was measured using the "rocket" method by Laurell [7] (the assay ranged between 20 and 200 μg/ml). The permeability index, i.e., albumin to total protein ratio, was calculated.

Pulmonary function

Positive response was defined as >20% fall in FEV_1 from baseline. Bronchial response was measured by a serial monitoring of forced expiratory volume in one second (FEV_1) using a spirometer (Vicatest 2A, Mijnhardt, Holland) before and 5 min, 5 h and 24 h after the challenge.

Histamine dihydrochloride obtained from the Sigma Chemical Company was prepared in normal saline solution immediately before the inhalation and delivered through De Vilbiss nebulizer No. 464. The bronchial challenge with histamine was performed before and 24 h after the challenge. Histamine concentrations were as follows: 0.03; 0.06; 0.125; 0.250; 0; 1; 2; 4; 8 and 16 mg/ml. Histamine PC_{20} FEV_1 is defined as the provocation concentration causing a 20% fall in FEV_1 [8].

Rhinomanometry

Rhinomanometry (RMM) was performed at the following time-points: immediately before the provocation, directly 1, 2, 3, 4, 5, 6 and 24 h after provocation with placebo and occupational allergens, respectively. A Mes rhinomanometer (Mes Co., Poland) was used. We measured the expiratory nasal resistance (ENR), as these curves are reported to be less variable from breath to breath than the inspiratory curves.

RMM was considered positive if the ENR value increased about threefold above the baseline.

Statistical analysis

Cell counts and the level of albumin were compared with the basal values using the Wilcoxon matched pairs signed-rank test. The data were expressed as the mean ± SD. The results obtained after specific challenge in patients with occupational allergy, in healthy subjects and atopic patients with non-occupational allergy were compared using the Mann-Whitney U test. The differences were regarded as significant at $p < 0.05$.

The relations between parametric data, such as the percentage of cells and ENR were analyzed by Pearson's correlation test, and between non-parametric data (such as the symptom score values) and parametric data by the Spearman rank correlation test.

RESULTS

Clinical and immunological findings are presented in Tables 1, 2 and 3. In the group of occupational allergics the following was found: the mean total IgE was 134 KU/l; specific IgE to occupational allergens was detected in 8 of the 25 patients and 13 subjects had positive SPTs to laboratory animal allergens.

Table 1. Clinical and immunological findings in subjects with occupational airway allergy

Parameter	Subjects exposed to occupational allergens (n = 25)
Mean age (yr)	41.9 ± 11.6
Family history of atopy	18
Family history of allergic diseases	10
History of respiratory disease	4
Allergic symptoms specific for bronchial asthma	25
Allergic symptoms specific for allergic rhinitis	25
Smoking habits:	
non-smoker	17
ex-smoker	3
heavy smoker	5
Mean duration of occupational exposure	13 ± 7
Total IgE level	134 ± 28
Positive RAST	8
Positive SPTs to common allergens	18
Positive SPTs to laboratory animal allergens	13

Data are shown as mean ± SD.

Table 2. Clinical and immunological findings in subjects with non-occupational allergy

Parameter	Subjects with no occupational exposure (n = 22)
Mean age (yr)	45.5 ± 8.2
Family history of atopy	16
Family history of allergic diseases	11
History of respiratory disease	9
Allergic symptoms specific for bronchial asthma	22
Allergic symptoms specific for allergic rhinitis	22
Smoking habits:	
non-smoker	14
ex-smoker	6
heavy smoker	2
Mean duration of exposure to occupational allergens	0
Total IgE level	105 ± 10
Positive RAST to laboratory animal allergens	0
Positive SPTs to house dust mite	22
Positive SPTs to other common allergens	8
Positive SPTs to laboratory animal allergens	0

Data are shown as mean ± SD.

Table 3. Clinical and immunological findings in healthy subjects

Parameter	Subjects with no occupational exposure (n = 15)
Mean age (yr)	42.9 ± 10.4
Family history of atopy	0
Family history of allergic diseases	2
History of respiratory disease	2
Allergic symptoms specific for bronchial asthma	0
Allergic symptoms specific for allergic rhinitis	0
Smoking habits:	
non-smoker	8
ex-smoker	4
heavy smoker	3
Mean duration of exposure to occupational allergens	0
Total IgE level	65 ± 17
Positive RAST to laboratory animal allergens	0
Positive SPTs to house dust mite	5
Positive SPTs to other common allergens	3
Positive SPTs to laboratory animal allergens	0

Data are shown as mean ± SD.

Symptom score

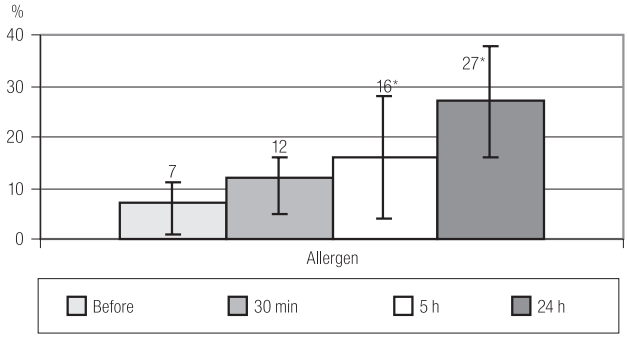
The provocation with laboratory animal allergens produced the symptoms of rhinitis in all patients with occupational allergy. These symptoms occurred immediately after the challenge and persisted for up to 24 h. All these patients displayed nasal symptoms, such as congestion, rhinorrhea and sneezing, with the mean symptom score amounting to 5.0 ± 1.0 immediately after the challenge, 4.0 ± 2.0 and 6.0 ± 1.0 after 5 and 24 h, respectively.

In 2 of the 25 patients with occupational asthma, placebo was also found to induce the symptoms of rhinitis, but only immediately after the challenge (2.0 ± 1.0). After allergen and placebo inhalations, none of the healthy subjects or patients with non-occupational allergy developed symptoms of rhinitis.

In the group of patients with occupational allergy, the total symptom score immediately, 5 and 24 h after specific challenge significantly correlated with ENR measured by rhinomanometry (Spearman $r = 0.38$; $r = 0.36$; $r = 0.39$, respectively; $p < 0.05$).

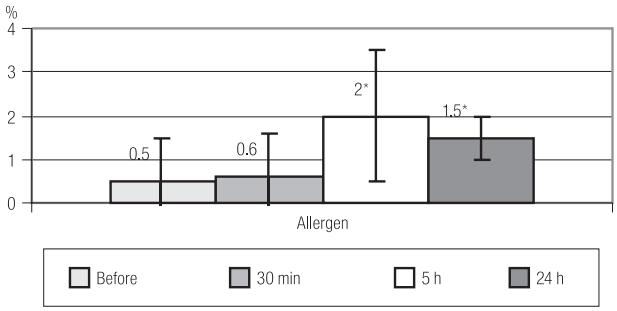
Cellular and biochemical findings in NALF after specific and placebo inhalations

All the patients with occupational allergy exhibited a significant increase in the percentage of eosinophils and basophils recovered from NALF 5 and 24 h after allergen inhalation (Figs. 1 and 2). There was no significant increase in the percentage of neutrophils in NALF at any time-point (Fig. 3).



* $p < 0.05$ vs basal value.

Fig. 1. Eosinophil changes in nasal washings of patients with occupational allergy after specific challenge.



* p < 0.05 vs basal value.

Fig. 2. Basophil changes in nasal washings of patients with occupational allergy after specific challenge.

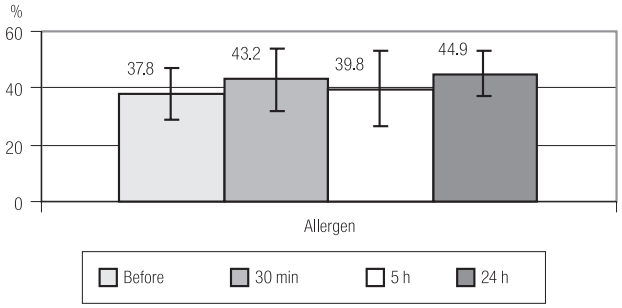
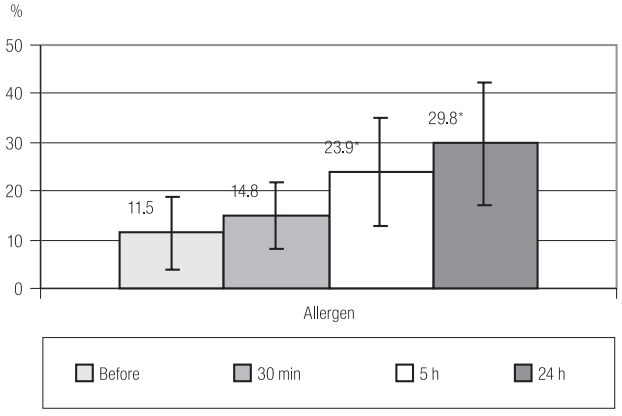


Fig. 3. Neutrophil changes in nasal washings of patients with occupational allergy after specific challenge.

Protein analysis of NALF from occupational allergics showed a significant increase in the albumin level 5 and 24 h after allergen challenge (Fig. 4).

After placebo inhalation, no morphological or biochemical changes were observed in NALF from patients with occupational allergy.



* p < 0.05 vs basal value.

Fig. 4. The level of albumin (mucosal permeability index) in nasal washings of patients with occupational allergy after specific challenge.

No significant increase was found in the percentage of eosinophils, basophils and neutrophils or in albumin level in NALF from healthy subjects and patients with non-occupational allergy at any time-point after specific and placebo inhalations.

In occupational allergics, the percentage of eosinophils and basophils in NALF correlated with ENS 5 and 24 h after the specific challenge ($p < 0.05$) (Table 4).

Table 4. Correlation coefficients between the proportion of eosinophils and basophils in nasal washings of subjects with occupational airway allergy and expiratory nasal resistance (ENR)

The proportion of:	ENR
Eosinophils	
5 h after specific challenge	0.47*
24 h after specific challenge	0.49*
Basophils	
5 h after specific challenge	0.40*
24 h after specific challenge	0.43*

* p < 0.05

Pulmonary function

A significant decrease in FEV_1 was observed in 17 of the 25 occupational allergics immediately, and in all occupational allergics 24 h after specific challenge (FEV_1 values were 3.9 ± 0.39 l/s before provocation; 2.7 ± 0.5 l/s immediately; 3.7 ± 0.33 l/s 5 h; and 1.9 ± 0.9 l/s 24 h after provocation ($p < 0.05$).

Within the period of observation, placebo inhalation did not induce significant changes in FEV_1 in patients with occupational allergy.

After specific challenge no significant changes in FEV_1 were found in the group of patients with non-occupational allergy (FEV_1 before provocation - 3.7 ± 0.23 l/s; immediately – 3.5 ± 0.24 l/s; 5 h – 3.6 ± 0.33 l/s; and 24 h after provocation – 3.9 ± 0.7 l/s; $p > 0.05$) or in healthy subjects (FEV_1 before provocation – 4.9 ± 1.23 l/s; immediately – 4.8 ± 0.24 l/s; 5 h – 5.1 ± 0.33 l/s; and 24 h after provocation – 4.69 ± 0.7 l/s; $p > 0.05$).

No marked changes in FEV_1 could be noted in healthy subjects or in non-occupational allergics after placebo inhalation.

Statistically significant differences in PC_{20} were found in the patients with occupational asthma after allergen provocation (2.9 ± 0.56 mg/ml before, and 1.2 ± 0.4 mg/ml 24 h after provocation).

No statistically significant differences in PC_{20} could be noted in non-occupational allergics and healthy subjects after specific challenge.

DISCUSSION

Allergic inflammation has been recognized as a feature of different allergic inflammatory diseases, such as bronchial asthma and allergic rhinitis. Occupational asthma and rhinitis caused by laboratory animal allergens are important health problems for subjects exposed to these high-molecular weight agents. The knowledge of factors responsible for the incidence of these diseases remains rather scarce, thus a number of researchers have been seeking new diagnostic methods to confirm the relationship between the occupational agent and the allergic symptoms.

In our opinion, a specific allergen challenge coupled with the monitoring of the proportion of different cells and the level of albumin seems to be the best method for differentiating between specific allergic and non-specific irritant reactions.

Typically, nasal allergen challenge induces a prolonged increase in eosinophil count and a less pronounced but very characteristic increase in metachromatic cell count [2,9–12]. Apart from these symptoms, we have also observed in the present study a prolonged and significant influx of eosinophils and basophils in NALF of patients with occupational allergy after specific challenge. This influx persisted for up to 24 h post-challenge, which suggests that these cells may be involved in the active inflammatory process that develops in the airway. Basophils may also be important during the late phase reaction [2,3,13]. Our data support the concept that basophils are recruited to the airway after allergen provocation.

We noticed no increase in neutrophil percentage in nasal washings after specific challenge. In our study, the percentage of eosinophils and basophils correlated with ENR during the late allergic reaction. The increase in the percentage of these cells was associated with the intensity of nasal obstruction 5 and 24 h after allergen challenge. Neither did we observe any significant changes in ENR and SS at any time-point after specific challenge in healthy subjects or in patients with non-occupational allergy. These findings confirm the major importance of nasal obstruction in the assessment of nasal early and late phase reaction [14,15]. In fact, both these clinical symptoms and RMM significantly correlated with the influx of inflammatory cells.

In the present study, the eosinophils displayed the most significant and persistent rise, as well as the most significant correlations with the clinical findings. Therefore, they are likely to play a crucial role in the alterations causing the nasal early and late phase reaction.

The present study has revealed that the prolonged increase in albumin/total protein ratio as an index of mucosal permeability is also specific for the allergic response [16,17]. In our previous studies we did not observe prolonged changes in the level of albumin after placebo and irritant inhalations [18,19].

In occupational asthmatics, the mean serum total IgE was 134 kU/l, significantly higher than in the group of healthy subjects. Therefore, we have drawn a conclusion similar to that of other authors that atopy is a factor predisposing to the development of allergy to laboratory animals [20]. There is also some evidence that atopy may shorten the latent period of the disease from individual exposure [21]. We noted the presence of specific IgE in 8 subjects of the 25 ones participating in the study. Our results are in agreement with other reports indicating that in almost 50% of subjects IgE is not detectable.

We did not find any significant difference in the smoking status between the three groups of subjects. Our results are similar to the findings of other authors who could not demonstrate convincing relationship between the smoking status and the development of laboratory animal allergy [20]. In the present study, we observed a significant decrease in FEV_1 both in the early and late phase response after specific challenge in most of the patients with atopic occupational asthma and rhinitis. This type of allergic reaction is specific for bronchial asthma caused by high molecular weight agents [22,23].

Conclusions

Our study demonstrated that:

- eosinophils and basophils are the predominant cells in NALF of patients with occupational airway allergy after a challenge with laboratory animal allergens;
- the inflammatory reaction constantly occurs after specific challenge and its intensity is related to the total symptom score and expiratory nasal resistance in occupational allergics;
- nasal lavage is a simple method that can be used for diagnosing occupational airway allergy in subjects occupationally exposed to high-molecular weight agents.

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