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Quantitative Cytology of Nasal Secretions and Scrapings in Japanese Cedar Pollinosis Patients

[Independent Papers]

Takeuchi, Kazuhiko MD; Ukai, Kotaro MD; Lee, Hwa Sik MD; Majima, Yuichi MD; Sakakura, Yasuo MD

From the Department of Otorhinolaryngology, Mie University School of Medicine, Tsu, Japan.
Editor's Note: This Manuscript was accepted for publication May 17, 1996.
Send Reprint Requests to Yasuo Sakakura, MD, Department of Otorhinolaryngology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514, Japan.

Abstract

To explore the dynamics of the cellular response to natural allergen exposure, the authors of this study performed quantitative cytology in both the nasal secretions and nasal scrapings of 16 normal control subjects and 23 Japanese cedar pollinosis patients before and during the pollen season. In nasal scrapings, the number of metachromatic cells increased significantly during the pollen season, although there was no significant difference between the normal subjects and the preseason pollinosis patients. The cell differential count in the patients with pollinosis showed that lymphocytes were predominant in scrapings; however, the majority of inflammatory cells in the nasal secretions were neutrophils before the season and eosinophils during the season. The study findings suggest that the appearance of metachromatic cells in nasal scrapings is specific to the pollen exposure and that the cytology in nasal secretions is significantly different from that in nasal scrapings.

INTRODUCTION

In allergic rhinitis, the nasal mucous membrane is infiltrated by inflammatory cells, including mast cells,[1] eosinophils,[2] basophils,[3] macrophages,[4] and lymphocytes.[5] Recently, it has been clarified that the infiltration of these inflammatory cells is caused by the expression of cytokines specific to each cell type. Moreover, the immunolocalization of cytokines and the expression of cytokine messenger RNAs (mRNAs) have been studied in allergic nasal mucosa. A significant increase of mRNA-producing cells for interleukin (IL)-3, IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) has been observed in the nasal mucosa after local allergen provocation.[6] Bradding, et al.[7,8] reported that mast cells are the major source of IL-4, IL-5, and IL-6. The recruitment of eosinophils during allergen-induced rhinitis may be associated with IL-3, IL-4, IL-5, and GM-CSF expression.

Seasonal rhinitis can serve as a good model for studying the cellular response in allergen reaction, because it is possible to compare the cytology before and during pollen exposure. Japanese cedar (*Cryptomeria japonica*) pollinosis is a common nasal disorder. Approximately 10% of the Japanese population show allergic reactions to this pollen, with symptoms occurring from February to April.[9] The present study was conducted to examine the changes in the cellular components of nasal secretions and scrapings before and during pollen exposure and to determine whether there is a difference in major cell type between nasal secretions and nasal scrapings. To quantify the individual cell types, we used a previously described cytocentrifuge technique.[10] We examined the cytology of nasal secretions and scrapings in both pollinosis patients and normal control subjects.

MATERIALS AND METHODS

Subjects

The study subjects included 23 patients with a history of rhinitis symptoms confined to the Japanese cedar pollen season (February through April), a positive skin test to the pollen extract, and a positive radioallergosorbent test (RAST) to the pollen. The patients

had no history of perennial allergy and had no positive RAST to house dust, house dust mite, *Candida*, or orchard grass (*Dactylis glomerata*). The 12 male and 11 female subjects ranged in age from 9 to 67 years, with a mean age of 34.7 years.

Sixteen normal healthy control subjects were also studied. These individuals had no history of allergies or nasal symptoms at any time of the year. Furthermore, they were nonsmokers and had negative skin tests to the Japanese cedar pollen. The 14 male and 2 female control subjects ranged in age from 22 to 26 years, with a mean age of 24.1 years.

Study Design

The patients were seen on two occasions. They first visited our clinic in October or November 1990, when they were free from nasal symptoms. At that time, both nasal secretions and nasal scrapings were obtained. Secretions were obtained from both nasal cavities by aspiration with Juhn Tym-Tap (Xomed-Treace Inc., Jacksonville, Fla.), and nasal scrapings were gently obtained from the inferior turbinate surface using a curette. The patients were asked to come to the clinic again when they first experienced nasal symptoms in February or March 1991 (i.e., the start of the Japanese cedar pollen season). Nasal secretions and scrapings were obtained again using the same procedure and without prior medication or treatment. Samples were also taken from normal control subjects before the Japanese pollen season.

Quantitative Cytology

The nasal secretions and scrapings were immediately diluted by the addition of a proportional amount of 0.067 mol/L phosphate-buffered saline (pH 7.2) containing 0.01 mol/L dithiothreitol [11] to obtain a final dilution of 10 to 100 times, depending on its consistency. The diluted sample was thoroughly shaken and was incubated at 4°C for 1 hour.

For quantitative cytologic analysis, smears of the diluted samples were prepared using a cytocentrifuge (Cytospin 2; Shandon

Southern Products Limited, Cheshire, U.K.). A 30-µL portion of the diluted sample was placed in each centrifuge chamber, and 200 µL of Hanks' balanced salt solution was added to each chamber to facilitate the diluted sample's transport. The cytocentrifugation was carried out at a speed of 1200 rpm for 10 minutes. The cytocentrifuge-prepared smears were rapidly air-dried and stained using the Wright-Giemsa method. The stained preparations were examined under oil immersion at a magnification of 3 1000.

For the nasal secretions, the absolute cell count of each cell type per cubic millimeter of the nasal secretion was calculated. The number of cells per cubic millimeter of nasal secretion (C_s) was calculated by the mean number of cells per microscopic field (C_m), the dilution factor for the nasal secretion (B), the amount (µL) of the diluted sample (A), the diameter of the cytocentrifuge-prepared smear (D_s), and the diameter of the microscopic field (D_m): $C_s = BC_mD_s^2/AD_m^2$.

For the nasal scrapings, the relative count of each cell type was calculated per 1000 epithelial cells. Both in nasal secretions and nasal scrapings, the cell differential count was determined in each sample as a percentage of 100, and then the mean and the standard error of the means (SEM) were calculated.

In this study, inflammatory cells include neutrophils, eosinophils, metachromatic cells, lymphocytes, and monocytes.

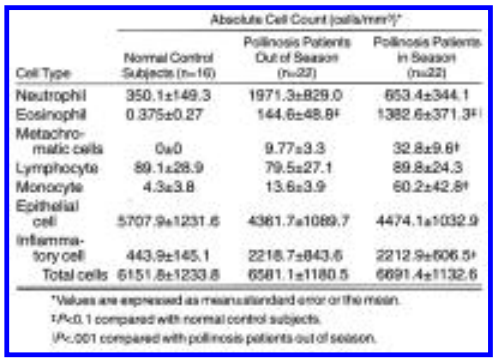
Statistical Analysis

The paired Wilcoxon's rank sum test was used to detect significant differences in absolute cell count, cell differential count, and relative cell count between the preseasonal count and the seasonal count. The unpaired Wilcoxon's rank sum test was used to detect significant differences in absolute cell count, cell differential count, and relative cell count between the allergic group and the normal subjects. The inflammatory cell correlation was determined by Spearman rank correlation. A *P* value of less than.05 was considered significant. The values are stated within the text as the mean and the SEM.

RESULTS

Nasal Secretions

Regarding the absolute cell count (Table I), only the number of eosinophils increased significantly during the pollen season in the allergic group. The number of eosinophils before the pollen season in the allergic group (SRo) was higher than in the normal control subjects (NC). The numbers of eosinophils, metachromatic cells, monocytes, and total inflammatory cells were significantly higher during the pollen season in the allergic group(SRi) than in the NC. There was no significant difference in the number of neutrophils among the SRo, SRi, and NCs. Regarding the cell differential count (Table II), the majority of inflammatory cells were eosinophils in the SRi and neutrophils in both the SRo and NC.



Cell Type	Absolute Cell Count (cells/mm³)* Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=22)	Pollinosis Patients In Season (n=22)
Neutrophil	350.1±149.3	1971.3±829.0	653.4±344.1
Eosinophil	0.375±0.27	144.6±48.8†	1382.6±371.3‡
Metachromatic cells	0±0	9.77±3.3	32.8±9.6†
Lymphocyte	89.1±28.9	79.5±27.1	89.8±24.3
Monocyte	4.3±3.8	13.6±3.9	60.2±42.8†
Epithelial cell	5707.9±1231.6	4361.7±1089.7	4474.1±1032.9
Inflammatory cell	443.9±145.1	2218.7±843.6	2212.9±606.5†
Total cells	6151.8±1233.8	6581.1±1180.5	6691.4±1132.6

*Values are expressed as mean±standard error of the mean.
†P<0.1 compared with normal control subjects.
‡P<.001 compared with pollinosis patients out of season.

TABLE I. Absolute Cell Count in Nasal Secretions.

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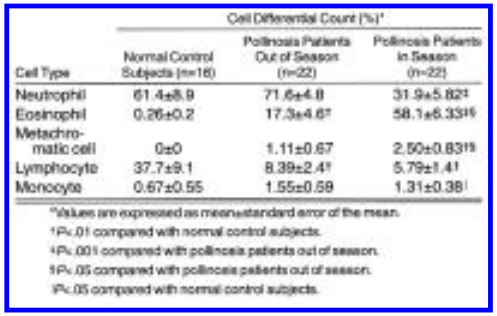

Cell Type	Cell Differential Count (%)* Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=22)	Pollinosis Patients in Season (n=22)
Neutrophil	61.4±8.9	71.6±4.8	31.9±5.82‡
Eosinophil	0.26±0.2	17.3±4.6†	58.1±6.33‖§
Metachromatic cell	0±0	1.11±0.67	2.50±0.83‖§
Lymphocyte	37.7±9.1	8.39±2.4†	5.79±1.4†
Monocyte	0.67±0.55	1.55±0.59	1.31±0.38‖

*Values are expressed as mean±standard error of the mean.
†P<.01 compared with normal control subjects.
‡P<.001 compared with pollinosis patients out of season.
§P<.05 compared with pollinosis patients out of season.
‖P<.05 compared with normal control subjects.

TABLE II. Cell Differential Count in Nasal Secretions.

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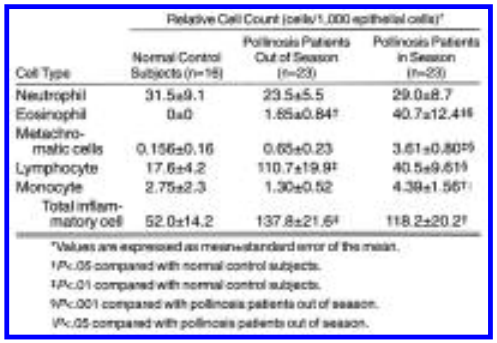
Nasal Scrapings

The relative cell count and cell differential count are listed in Tables III and IV, respectively. The numbers of eosinophils, metachromatic cells, and monocytes were significantly higher in the SRi than in the SRo. The number of lymphocytes was significantly lower in the SRi than in the SRo. The number of eosinophils and lymphocytes in the SRo was significantly higher than in the NC. The numbers of eosinophils, metachromatic cells, monocytes, and total inflammatory cells were significantly higher in the SRi than in the NC. No significant difference was observed in the number of neutrophils among the SRo, SRi, and NC. Regarding the cell differential count, the majority of inflammatory cells were neutrophils in the NC and lymphocytes in both the SRi and the SRo.



Cell Type	Relative Cell Count (cells/1,000 epithelial cells)* Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=23)	Pollinosis Patients In Season (n=23)
Neutrophil	31.5±9.1	23.5±5.5	29.0±8.7
Eosinophil	0±0	1.65±0.84†	40.7±12.4‖¶
Metachromatic cells	0.156±0.16	0.65±0.23	3.61±0.80‖§
Lymphocyte	17.6±4.2	110.7±19.9‡	40.5±9.6‖§
Monocyte	2.75±2.3	1.30±0.52	4.38±1.56†‖
Total inflammatory cell	52.0±14.2	137.8±21.6‡	118.2±20.2‡

*Values are expressed as mean±standard error of the mean.
†P<.05 compared with normal control subjects.
‡P<.01 compared with normal control subjects.
§P<.001 compared with pollinosis patients out of season.
‖P<.05 compared with pollinosis patients out of season.

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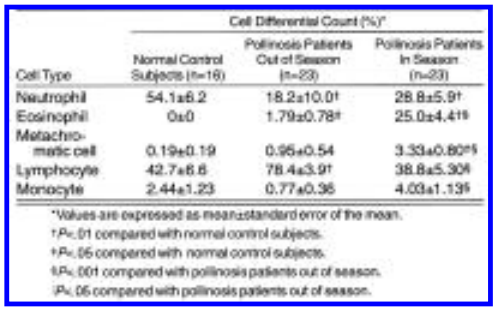
TABLE III. Relative Cell Count in Nasal Scrapings.



Cell Type	Cell Differential Count (%)* Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=23)	Pollinosis Patients In Season (n=23)
Neutrophil	54.1±6.2	18.2±10.0†	28.8±5.9†
Eosinophil	0±0	1.79±0.78†	25.0±4.4‖¶
Metachromatic cell	0.19±0.19	0.95±0.54	3.33±0.80‖§
Lymphocyte	42.7±6.6	78.4±3.9†	38.8±5.9‖§
Monocyte	2.44±1.23	0.77±0.36	4.03±1.13§

*Values are expressed as mean±standard error of the mean.
†P<.01 compared with normal control subjects.
‡P<.05 compared with normal control subjects.
§P<.001 compared with pollinosis patients out of season.
‖P<.05 compared with pollinosis patients out of season.

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TABLE IV. Cell Differential Count in Nasal Scrapings.

During the season, the number of eosinophils correlated significantly with the number of metachromatic cells (*rs*=.636, *P*<.005), lymphocytes (*rs*=.627, *P*<.005), and monocytes (*rs*=.5284, *P*<.01) but not with the number neutrophils (*rs*=.0962). Neutrophils were not correlated with metachromatic cells (*rs*=.0638), lymphocytes (*rs*=-.1175), or monocytes (*rs*=-.103). Lymphocytes were positively correlated with metachromatic cells (*rs*=.590, *P*<.005), and monocytes (*rs*=.456, *P*<.025).

DISCUSSION

Immediate allergic nasal symptoms of itching, sneezing, and watery rhinorrhea occur as a consequence of the immunoglobulin E (IgE)-dependent activation of mast cells in the nasal mucosa. The appearance of mast cells in the nasal mucosal epithelium has been reported in patients with allergic rhinitis after allergen provocation [12] and during the pollen season.[13,14] In the present study, the number of metachromatic cells in the allergic epithelial scrapings significantly increased during the pollen season, although the number of metachromatic cells in scrapings did not differ significantly between the NC and the SRo. This indicates that the appearance of metachromatic cells in the nasal scrapings is specific to the exposure to the cedar pollen, but not to its sensitization.

We believe that most of the metachromatic cells in nasal scrapings are mast cells, because Otsuka, et al.[15] reported that more than 80% of the metachromatic cells in nasal scrapings were mast cells, almost exclusively of the atypical "mucosal" type, which has recently been termed the"tryptase-positive, chymasenegative mast cell" (MC_T).[16] According to Pipkorn, et al.,[17] mast cells start to appear 4 or 5 days after pollen exposure. Bentley, et al.[14] also reported that mast cell counts revealed seasonal increases in MC_T but not chymase- plus tryptase-positive mast cells (MC_{TC}). The mechanism of mast cell appearance is not fully understand, but the animal experiment by Kawaguchi, et al.[18] showed that mast cells migrate into intraepithelial spaces from the subepithelial connective tissue stroma upon exposure to allergens.

The number of metachromatic cells in nasal secretions was significantly higher in the SRi than in the NC. Our previous study [10] using different groups of subjects indicates that the number of basophilic cells is significantly higher in patients with perennial allergic rhinitis than in normal control subjects and that metachromatic cells in nasal secretions are predominantly basophils.[15]

The appearance of eosinophils is important in allergic rhinitis, because eosinophils have been observed in the nasal secretions of 100% of patients with perennial and seasonal allergic rhinitis and in only 11.8% in normal control subjects.[10] In the present study, there was significant differences in the eosinophil counts in the three groups (NC<SRo<SRi), both in nasal secretions and in nasal

scrapings. Eosinophils are recruited into nasal mucosa by IL-3, IL-4, IL-5, and GM-CSF produced by T lymphocytes or mast cells via Ig-E dependent stimulation.[6,7] This could explain the increase of eosinophils during the pollen season.

The appearance of eosinophils before the pollen season in the allergic patients may be due to the existence of lymphocytes ($CD25^+$) in SRo,[5] or the patients could be allergic to other pollens or molds that cause allergic reactions. The positive correlation between metachromatic cells and eosinophils in nasal scrapings suggests that infiltration of these cells is regulated in a similar manner or that one cell type may recruit the other. Cytokines produced and released by mast cells [7] may recruit eosinophils.

In the present study, there was no significant difference in the number of neutrophils among the three groups, either in nasal secretions or in nasal scrapings. This is in agreement with previous reports [10,14] and indicates that neutrophils are not involved in the pathophysiology of allergic rhinitis.

The reason that the lymphocyte counts decreased in nasal scrapings during the season is unknown. Although the most numerous inflammatory cells in allergic nasal scrapings are lymphocytes, little information is available on the extent and nature of infiltration of the nasal mucosa by lymphocytes in allergic rhinitis and how this infiltrate might vary with exposure to allergen. Calderón, et al.[5] reported that the total T cell number ($CD3^+$ T cells) was increased during the pollen season both in the nasal epithelium and in the lamina propria. On the other hand, Bentley, et al.[14] observed no statistically significant changes within the submucosa or the epithelium for total leukocytes ($CD45^+$ cells) or T lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, and $CD25^+$ cells).

Although the majority of inflammatory cells in both the nasal secretions and scrapings of normal control subjects were neutrophils, there was a discrepancy between the cytology of the nasal secretions and the nasal scrapings in the pollinosis group. The cell differential count in the patients with pollinosis showed that lymphocytes were predominant in scrapings but that the majority of the

inflammatory cells in the nasal secretions were neutrophils before the season and eosinophils during the season.

The recruitment of eosinophils into nasal secretions during the pollen season could be explained by the expression of cytokines and cell adhesion molecules. Montefort, et al.[19] reported that the expression of both intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 (VCAM-1) was increased in patients with perennial rhinitis compared with nonatopic healthy volunteers. Fukuda, et al.[20] reported that the number of eosinophils correlated significantly correlated with the IL-4 level in bronchial lavage fluid in asthmatic patients and that IL-4-positive asthmatics showed a significantly increased expression of VCAM-1 as compared with both IL-4-negative asthmatic patients and control subjects. Therefore, it is probable that the expression of IL-4 and the VCAM-1 level increased on exposure to pollens in the patients with pollinosis and that this resulted in the preferential migration of eosinophils into the airways.

The predominance of neutrophils in the nasal secretion of both the NC and the SRo could be explained by the expression of IL-8. It is known that IL-8 is constitutively expressed by human nasal epithelium and that its expression is readily increased upon virus infection or upon stimulation with IL-1 and tumor necrosis factor.[21] Both eosinophils and neutrophils may play a role after they are recruited into nasal secretions.

CONCLUSION

The number of eosinophils is increased, even out of the pollen season, in the pollinosis patients, and the appearance of metachromatic cells in the nasal scrapings is specific to the pollen exposure. The major cell types in nasal scrapings and nasal secretions differ significantly.

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TABLE I

Cell Type	Absolute Cell Count (cells/mm³)*		
	Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=22)	Pollinosis Patients in Season (n=22)
Neutrophil	350.1±149.3	1971.3±829.0	653.4±344.1
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*Values are expressed as mean±standard error or the mean.

‡$P<0.1$ compared with normal control subjects.

|$P<.001$ compared with pollinosis patients out of season.

TABLE I. Absolute Cell Count in Nasal Secretions.
From: Takeuchi: Laryngoscope, Volume 106(11).November 1996.1419-1423

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TABLE II

Cell Type	Cell Differential Count (%)*		
	Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=22)	Pollinosis Patients in Season (n=22)
Neutrophil	61.4±8.9	71.6±4.8	31.9±5.82‡
Eosinophil	0.26±0.2	17.3±4.6†	58.1±6.33‡§
Metachromatic cell	0±0	1.11±0.67	2.50±0.83†§
Lymphocyte	37.7±9.1	8.39±2.4†	5.79±1.4†
Monocyte	0.67±0.55	1.55±0.59	1.31±0.38‖

*Values are expressed as mean±standard error of the mean.

†$P<.01$ compared with normal control subjects.

‡$P<.001$ compared with pollinosis patients out of season.

§$P<.05$ compared with pollinosis patients out of season.

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TABLE III

Cell Type	Relative Cell Count (cells/1,000 epithelial cells)* Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=23)	Pollinosis Patients in Season (n=23)
Neutrophil	31.5±9.1	23.5±5.5	29.0±8.7
Eosinophil	0±0	1.65±0.84†	40.7±12.4‡§
Metachromatic cells	0.156±0.16	0.65±0.23	3.61±0.80‡§
Lymphocyte	17.6±4.2	110.7±19.9‡	40.5±9.61§
Monocyte	2.75±2.3	1.30±0.52	4.39±1.56†‖
Total inflammatory cell	52.0±14.2	137.8±21.6‡	118.2±20.2†

*Values are expressed as mean±standard error of the mean.

†$P<.05$ compared with normal control subjects.

‡$P<.01$ compared with normal control subjects.

§$P<.001$ compared with pollinosis patients out of season.

‖$P<.05$ compared with pollinosis patients out of season.

TABLE III. Relative Cell Count in Nasal Scrapings.
From: Takeuchi: Laryngoscope, Volume 106(11).November 1996.1419-1423

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TABLE IV

Cell Type	Cell Differential Count (%)*		
	Normal Control Subjects (n=16)	Pollinosis Patients Out of Season (n=23)	Pollinosis Patients In Season (n=23)
Neutrophil	54.1±6.2	18.2±10.0†	28.8±5.9†
Eosinophil	0±0	1.79±0.78‡	25.0±4.4†§
Metachromatic cell	0.19±0.19	0.95±0.54	3.33±0.80†§
Lymphocyte	42.7±6.6	78.4±3.9†	38.8±5.30§
Monocyte	2.44±1.23	0.77±0.36	4.03±1.13§

*Values are expressed as mean±standard error of the mean.

†$P<.01$ compared with normal control subjects.

‡$P<.05$ compared with normal control subjects.

§$P<.001$ compared with pollinosis patients out of season.

‖$P<.05$ compared with pollinosis patients out of season.

TABLE IV. Cell Differential Count in Nasal Scrapings.
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