Original Article

Changes in nasal symptoms and inflammatory cells over the course of perennial allergic rhinitis

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ABSTRACT
Nasal symptoms and inflammatory cells changes over the course of perennial allergic rhinitis have been analyzed only rarely. We studied nasal symptoms, nasal physical findings, and laboratory data in five groups, which consisted of varying time periods during the course of perennial allergic rhinitis (< 1, 1–2, 2–3, 3–5 and 5+ years) of 354 patients at the time of the first visit to our allergy clinic. The mean values calculated from scored nasal symptoms (i.e., rhinorrhea, sneezing and nasal obstruction) and physical findings (i.e., inferior turbinate swelling and colour) showed significant differences among these groups. We also studied inflammatory cells, mast cells, EG2+ and total eosinophils, CD3+ and CD4+ T cells, and neutrophils in the nasal mucosa of five patients with a short history (< 1.5 years) of allergic rhinitis, and of 10 patients with a long history (> 3 years). The tissue density of mast cells in the epithelial layer and of eosinophils and CD4+ T cells in the subepithelial layer did differ between the two groups. These results indicate that perennial allergic rhinitis goes through a transition stage from onset to a few years, and thereafter becomes a chronic condition. Mast cells, eosinophils, and CD4+ T cells may be associated with ongoing allergic inflammation.

Key words: CD4+ T cells, eosinophils, mast cells, nasal findings, nasal mucosa, nasal symptoms, perennial allergic rhinitis.

INTRODUCTION
Patients with typical perennial allergic rhinitis have daily repeated attacks of sneezing, watery rhinorrhea, and nasal obstruction. In general, the inferior turbinate of their nasal mucosa is pale and swollen, and serous rhinorrhea is detected in the nasal cavity. The pathophysiology of the onset of allergic rhinitis has been studied with natural pollen exposure and also with nasal challenge to pollen out of season.1,2 These reports studied the patients over a short time. However, patients with perennial allergic rhinitis continue to inspire a small amount of the exogenous allergen, such as house dust mite, every day. Changes in the nasal symptoms of such patients have been reported in a retrospective study,3 but nasal symptoms and inflammatory cells have not been studied at different points in the disease course.4 In this study, we checked the duration of allergic nasal symptoms when patients with allergic rhinitis visited our allergy clinic in the Department of Otolaryngology. We studied the relationship between the time-course of allergic rhinitis and the degree of nasal symptoms, nasal physical findings, and laboratory data in 354 perennially allergic patients whose allergen was house dust mite. Furthermore, to study the relationship between inflammatory cells and chronic allergic inflammation of the nasal mucosa, we investigated the tissue density of mast cells (tryptase+ cells), eosinophils, and their secreted type (EG2+ cells), CD3+ and CD4+ T cells, and neutrophils (elastase+ cells) in the nasal mucosa of patients with symptom duration of less than 1.5 years (short history) and of more than 3 years (long history).

MATERIALS AND METHODS
Clinical study
The subjects were 354 patients (186 men and 168 women) who visited our allergy clinic in the Department of Otolaryngology from 1986 to 1995. The patients
were assigned to groups according to the duration of their disease at the time of the first visit: <1, 1–2, 2–3, 3–5, and 5+ years. The number of patients in each group was 68, 56, 51, 63, and 116, respectively (Fig. 1). No significant differences of age distribution (<10, 11–20, 21–31, and 31+ years old) existed among these groups, except for the 5+ year group, which had only 10 patients younger than 10 years of age (Chi-squared test). All patients had common perennial rhinitis symptoms and eosinophilia in their nasal smears, determined by Hansel staining. The allergen causing allergic symptoms was house dust mite only, as confirmed by intradermal skin test responses. Skin tests for common allergens were performed: house dust, molds (Alternaria, Aspergillus and Penicillium), pollens (birch, orchard grass and timothy grass), and mugwort (Torii & Co. Ltd, Tokyo, Japan). We excluded patients who had shown an exacerbation of nasal symptoms in a limited season and had a positive skin test to pollen or mold. A great majority of the subjects had a positive nasal provocation test to house dust (2–5 μg protein nitrogen/disc, 7–8 μg total nitrogen/disc, Torii & Co. Ltd). In some negative subjects, we applied two discs to each nasal mucosa to confirm the positive response to house dust. A radioallergosorbent test (RAST) to house dust (Greer Lab Inc., Lenoir, USA) and Dermatophagoides farinae (D. farinae) was performed. We excluded patients who were being or had been treated with immunotherapy, or who had taken medicine for allergic rhinitis within 1 month prior to visiting. Patients with nasal polyps were also excluded; patients with asthma or atopic dermatitis either during or prior to the study were included.

Patients recorded the degree of nasal symptoms on daily diary cards for 1 week before visiting the allergy clinic. Patients with perennial allergic rhinitis do not necessarily have the same nasal symptoms all the year around. However, patients in this study visited our clinic at all seasons. The number of patients in each group was more than 51, so we judged it possible to study the difference between groups by nasal symptoms for each week. Nasal obstructions were scored on an arbitrary 4-point scale (0 = absent, 1 = mild, 2 = moderate and 3 = severe) according to Okuda’s criteria. The point for nasal obstruction, the number of sneezing attacks and the incidence of nasal blowing, which might indicate watery rhinorrhea, were recorded three times per day. The mean values of these symptoms per day were analyzed between each group (Fig. 2). Swelling of inferior turbinate was rated from 0 to 3 points in each nasal cavity. The inferior turbinate was classified from normal, slightly red, red, partly pale, or pale in color. Radioallergosorbent test and total IgE in serum and eosinophils in peripheral blood was expressed as scores, IU/ml, and /μL, respectively. The opacification of paranasal sinuses was judged with Waters’ and Caldwell’s views of X-ray.

**Immunohistochemical study**

The nasal mucosa of the inferior turbinate was obtained by a turbinectomy performed on those patients who desired the alleviation of nasal symptoms. Moreover, no patient took medicine for allergic rhinitis for 2 weeks before surgery. Five patients (mean age 32 years, range 12–52) had suffered from nasal symptoms for less than 1.5 years (short history) and 10 patients (mean age 28 years, range 13–51) had suffered for more than 3 years (long history). Although the degree of nasal symptoms, such as sneezing and nasal blowing, was almost the same in both groups, that of nasal obstruction was higher in patients with a long history than with a short history (P < 0.05, two-tailed Student’s t-test). The nasal mucosa, 10–20 mm from the anterior edge of the inferior turbinate, was sectioned and fixed with neutral buffer formalin for 1 day. Specimens were dehydrated and then embedded in paraffin. Three-micrometer sections were mounted on aminopropyltriethoxysilane-coated slides. The antigen retrieval was treated with 0.1% trypsin in 0.1% calcium chloride
(pH 7.6) for 10 min at 37°C to detect tryptase, or with a pressure cooker using 1 mmol/L EDTA (pH 8.0) instead of 0.01 mol/L citric acid for 1 min to detect CD3 and CD4.  

After blocking endogenous peroxidase, the sections were incubated with 10% heated inactivated rabbit serum, and then incubated with murine monoclonal antibodies or negative control (Table 1). The EG2 antibody was incubated for 2 h and the other antibodies for 1 h, at 37°C. Immunohistochemistry was performed by the labeled streptavidin-biotin method. Briefly, biotinylated rabbit IgG to mouse IgG (1/300, Dako, Copenhagen, Denmark) as a second step, and peroxidase-conjugated streptavidin (1/300, Dako, Copenhagen, Denmark) as a third step, were incubated for 1 h each. For the EG2-staining, alkaline-conjugated streptavidin (1/300, Dako, Copenhagen, Denmark) was performed. EG2+ cells were stained blue by naphthol AS-MX phosphate containing fast red BB, and the other cells were stained red by amino-ethylcarbazol containing H2O2. Except for the EG2-staining, counterstaining was performed with hematoxylin. To detect eosinophil without blue-colored EG2+ cells, sections were stained with eosin and then counterstained with methyl green.

**Assessment of tissue density of the cells**

We observed two zones of the nasal mucosa: the epithelial layer and the subepithelial layer of the lamina propria, which exists between the basal membrane and the underlying 220 μm. The latter was done using an eyepiece graticule (area: 0.22 mm × 0.33 mm). All positive cells in each area were counted. The area of the epithelial layer was measured with an Olympus computer image analysis (Rize Inc., Sendai, Japan). The tissue density of each positive cell in both the epithelial and subepithelial layers was calculated (per mm2).

**Table 1.** Murine monoclonal antibodies used in the present study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Detected cell</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptase</td>
<td>Chemicon</td>
<td>Mast cell</td>
<td>1:1000</td>
</tr>
<tr>
<td>EG2</td>
<td>Pharmacia</td>
<td>Secreted type of eosinophil</td>
<td>1:20</td>
</tr>
<tr>
<td>CD3</td>
<td>Novostra</td>
<td>T-cell</td>
<td>1:50</td>
</tr>
<tr>
<td>CD4</td>
<td>Novostra</td>
<td>Helper/inducer T-cell</td>
<td>1:20</td>
</tr>
<tr>
<td>Elastase</td>
<td>Dako</td>
<td>Neutrophil</td>
<td>1:100</td>
</tr>
<tr>
<td>IgG1</td>
<td>IC Biomed</td>
<td>Negative control</td>
<td>10 μg/mL</td>
</tr>
</tbody>
</table>

Fig. 2 Relationship between duration of allergic nasal symptoms and nasal symptoms. (a) Degree of nasal obstruction (□, 0–0.5; △, 0.6–1.5; □, 1.6–2.5; ■, 2.6–3); (b) incidence of nasal blowing per day (□, 0; △, 1–5; □, 6–10; ■, 11+); and (c) number of sneezing attacks per day (□, 0; △, 1–5; □, 6–10; ■, 11+). Statistically significant difference (P < 0.05) is indicated between the group with a history of less than 1 year and that of the shortest history in the groups of more than 2 years.
Statistical analysis
The nasal symptoms, nasal physical findings, and laboratory data were statistically compared by Chi-squared test, and tissue density data were analyzed using two-tailed Student's t-test. Significant difference was less than 0.05.

RESULTS
Nasal symptoms
Figure 2 shows the degree of each nasal symptom in each group. Regarding nasal obstruction, no significant difference existed between groups with a history of less than 3 years; however, a significant difference was found between those with a history of less than 1 year and those with a history of more than 3 years (Fig. 2a). There was a difference in the rate of nasal blowing between the less than 1 year group and the more than 2 year group (Fig. 2b). The number of sneezing attacks differed significantly only between the less than 1 year group and the more than 5 year group (Fig. 2c).

Nasal findings and laboratory data
As to the swelling and color of the nasal mucosa, there was a significant difference between the less than 1 year group and all other groups (Fig. 3a, b), but there was no significant difference between the various groups with a more than 2 year history. There were no differences between groups in the RAST score to D. farinae (Fig. 3c) and house dust. Patients with more than 1000 IU/mL of IgE in serum occasionally had atopic dermatitis and/or asthma. The rate of patients with those diseases was not different between each group (Fig. 1, parentheses). As

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**Fig. 3** Relationship between duration of allergic nasal symptoms and nasal findings. (a) Degree of nasal inferior turbinate mucosa swelling (○, 0–2.0; □, 2.1–2.9; △, 3.0–3.9; ■, 4.0–4.9; ■, 5.0+); (b) color of nasal inferior turbinate mucosa (●, normal; ○, light red; ■, red; △, partly pale; □, pale); (c) score of RAST to D. farinae (○, 0; ●, 1; △, 2; □, 3; ■, 4; ■, 5, 6); and (d) total IgE in serum (○, 0<300; ●, 300–500; △, 501–1000; ■, 1001 + IU/mL). The value (P < 0.05) is the same as in Fig. 2.
some patients who had more than a 5 year history without contracting those diseases showed a high IgE level, a significant difference appeared between the more than 5 year group and the other groups (Fig. 3d). Eosinophils in peripheral blood showed no significant difference between the groups. About 30% of each group had opacification in the paranasal sinuses, indicating no significant difference between the groups.

**Inflammatory cells in the nasal mucosa**

Figure 4 indicates the tissue density of inflammatory cells. Mast cells in the epithelial layer were more prominent in the nasal mucosa of the group with a long history than in the group with a short history (Figs. 4a, 5, A-a, A-b). Most eosinophils in the nasal mucosa were EG2-positive, with an average value of 97.7%. The tissue density of eosinophils was higher in the subepithelial layer than in the epithelial layer. Eosinophils in the subepithelial layer were more prominent in the mucosa of the group with a long history than in the group with a short history (Figs 4b, 5, B-a, B-b). Although there was no significant difference between each group in CD3+ T cells (Fig. 4c), CD4+ T cells in the subepithelial layer showed a significant difference between short and long history patients (Figs 4d, 5, C-a, C-b). Neutrophils were scattered sparsely in the nasal mucosa of both groups, with no significant difference between them (Fig. 4e).

**DISCUSSION**

The mechanism of the development of nasal symptoms and inflammatory cell infiltration during the course of perennial allergic rhinitis had not been reported previously. It is also unclear when the nasal mucosa of these patients changes to show the characteristic features of allergic rhinitis: the swelling and pale color of the inferior turbinate. These features are thought to be the result of vasodilation and tissue edema. This study showed that the nasal mucosa might gradually change over the first few years after the onset of allergic rhinitis. The degree of nasal symptoms, such as nasal obstruction, almost coincided with the change in nasal findings, such as swelling and color of the nasal...
mucosa. Sneezing and watery rhinorrhea induced by glandular secretion are associated with the nasal nerve reflex following the reaction of allergen and antibodies. Our results suggested that neural reflex activity and hyper-reactive nasal mucosa were increasing over the few years from the onset of perennial allergic rhinitis. The lack of change in RAST score indicates that after the onset of allergic rhinitis symptoms, the continued
inspiration of small amounts of allergen such as house dust mite does not produce increases in specific IgE. It is likely that the rate of specific IgE production to house dust and *D. farinae* was dependent upon individual characteristics of the patient’s circumstances and histocompatibility. In addition, the incidence of sinusitis did not differ between groups.

The total IgE level in the serum of patients with allergic rhinitis is generally low. Patients with both allergic rhinitis and a high IgE level are considered to have or to have had atopic dermatitis and/or asthma. The incidence of patients contracting these diseases showed no difference between each group in this study. Some patients with more than a 5-year history showed a high IgE level, the reason for which can not be explained at this time.

Although only five nasal mucosae were studied in the group with a short history, the tissue density of mast cells, eosinophils and CD4+ T cells showed a statistically significant difference between the two groups.

Chemical mediators from eosinophils reportedly relate to the late phase of nasal reaction after allergen challenge. Eosinophils, EG2+ cells in the nasal mucosa, increased after the challenge of pollen and were associated with mRNA of several cytokines, especially cytokines of the Th2 type. This increase of eosinophils in the nasal mucosa of the group with a long history may be due to the fact that some eosinophils may remain and survive in nasal mucosa at continuous allergen exposure, which may induce some cell-derived cytokines. T cells were also reported to be one source of IL-5 mRNA in allergen-induced rhinitis. The mechanism of increase in T cells may be similar to that underlying the in vitro proliferation observed in response to stimulation with specific allergen in patients with allergic rhinitis. The increase of EG2+ eosinophils and CD4+ T cells in the nasal mucosa of patients with a long history of allergic rhinitis may provide the relation to the pathological feature of chronic allergic inflammation. The accumulation of mast cells in the epithelial layer has been recognized in allergic rhinitis and is associated with the nasal symptoms. Mast cells in the perennial allergic nasal mucosa were reported to be a source of IL-4, which might be important for allergic inflammation. Mast cells proliferate in allergic nasal mucosa, and we recently reported that c-kit receptor-positive, tryptase-negative, IgE-negative cells are likely candidates for mast cell progenitors. Nasal mucosa in long-term allergic rhinitis has been suggested as providing a micro-environment in which mast cells have cytokines and increase in number. We confirmed that neutrophils were seldom associated with chronic allergic inflammation of nasal mucosa.

In conclusion, judging from changes in nasal symptoms and nasal physical findings, the time-course of perennial allergic rhinitis goes through a transition stage for the first few years after onset, subsequently being transformed to the chronic pathophysiologic stage. We also suggest that mast cells, eosinophils, and CD4+ T cells are associated with the pathological constitution of chronic allergic rhinitis.

**ACKNOWLEDGEMENT**

This work was supported in part by Grants-in-Aid for Scientific Research, administered by the Ministry of Education, Science, Sports and Culture, Japan (C-06671701).

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