ORIGINAL CONTRIBUTION

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Relationship between epithelial damage or basement membrane thickness and eosinophilic infiltration in nasal polyps with chronic rhinosinusitis*

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SUMMARY

Background: *Chronic rhinosinusitis (CRS) with nasal polyps is characterized by eosinophilic infiltration. This study hypothesized that the aggregation of the mucosal pathology during remodeling is related to infiltrating eosinophils in patients with such nasal polyps.*

Object: *To clarify the pathogenetic role of eosinophils in patients with CRS with nasal polyps, this study investigated the relationship between epithelial damage or basement membrane (BM) thickening and the epithelial infiltration of eosinophils in these nasal polyps.*

Methods: *The number of eosinophils that infiltrated into the epithelial and subepithelial layers of sinonasal tissues was counted. The staging of epithelial damage allowed the quantification of epithelial loss.*

Results: *Both epithelial damage and BM thickness in CRS, which were correlated with the number of infiltrated eosinophils, were significantly greater than in the control group. Neither parameter showed significant differences between the asthma and non-asthma groups. There was a significantly correlation in the eosinophilic infiltration between the subepithelial and epithelial layers.*

Conclusion: *It is suggested that eosinophils that infiltrate into both the epithelial and subepithelial layers play a part in the process of mucosal remodeling of CRS with nasal polyps.*

Key words: nasal polyps, chronic rhinosinusitis, epithelial damage, basement membrane thickness

INTRODUCTION

Chronic rhinosinusitis (CRS) is defined as persistent inflammation of the nasal and paranasal cavity mucosa persisting for at least 3 months [(1)]. An epidemiological study performed in the United States revealed that approximately 16% of the population has CRS. The prevalence and medical costs of CRS are increasing and have become an important social issue [(2)].

Although CRS is a multifactorial disease and a heterogeneous group of diseases, with different underlying etiologies and pathophysiologies, many published studies differentiate CRS without nasal polyps from CRS with nasal polyposis [(3-6)]. Patients with CRS without nasal polyps appear more likely to have signs of bacterial infection and have been reported to have a better response to medical treatment [(7)]. The phenotype of CRS without nasal polyps can be characterized by neutrophil recruitment into sinus effusion due to both upregulation of adhesion molecules of the vascular endothelium induced by interleukin (IL)-1 and enhanced secretion of the neutrophil chemoattractant, IL-8, from the epithelial cells and neutrophils [(8-11)].

The histomorphological pattern of CRS with nasal polyps is characterized by the predominance of eosinophils and mixed mononuclear cells and the relative paucity of neutrophils [(12)]. CRS with nasal polyps associated with mucosal infiltration with eosinophils may be regarded as eosinophilic CRS, due to the distinctive feature of tissue eosinophilia [(13)], which is more refractory to surgical treatment and is frequently associated with bronchial asthma. Several studies have reported a clinical relationship between CRS and asthma [(14-16)]. The cytokine profile in the sinus mucosa of CRS is similar to that in lung tissue of asthma [(17)]. The histopathological features of asthma, including the tissue eosinophils, epithelial damage and basement membrane (BM) thickening of the lower airway [(16,17)], are also observed in sinonasal specimens with CRS [(20)]. Epithelial dam-

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age in asthma is thought to be a consequence of ongoing inflammation, bronchoconstriction and host susceptibility factors [21-24]. The resultant epithelial damage is likely to trigger airway remodeling by releasing mitotic and fibrogenic growth factors from various inflammatory cells, particularly eosinophils, thus leading to deposition of collagen, angiogenesis and smooth muscle proliferation [25-27].

The present study was designed to evaluate the relationship between the eosinophilic accumulation in the sinonasal mucosa and upper airway remodeling in patients demonstrating CRS with nasal polyps.
Furthermore, the eosinophilic infiltration inside the epithelial layer, which is expected to be much more strongly related to the epithelial changes such as epithelial detachment and BM thickening, was compared to subepithelial eosinophilic accumulation. This study should provide a helpful key to clarify pathogenetic processes of intractable rhinosinusitis.

MATERIAL AND METHODS

Patients

This study included 45 patients with CRS with nasal polyps and 6 normal controls. Eighteen patients had bronchial asthma including 5 with aspirin-induced asthma. CRS was diagnosed based on the criteria of the European position paper [4]. The patients had two or more symptoms, one of which was either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), and/or facial pain/pressure, and/or reduction or loss of smell; and either endoscopic signs of polyps and/or mucopurulent discharge primarily from the middle meatus and/or oedema/mucosal obstruction primarily in the middle meatus and/or computed tomographic changes showing mucosal changes within ostiomeatal complex and/or sinuses. None of these patients were treated with either systemic corticosteroids or any other immune-modulating drugs. Any patient with CRS with nasal polyps associated with current signs of purulent nasal discharge, chronic obstructive pulmonary disease, diffuse panbronchiolitis, or fungal sinus disease, congenital mucociliary diseases, or cystic fibrosis was excluded from this study. All patients gave their written informed consent and the study was approved by the Ethics Committee of Juntendo University School of Medicine.

Sampling of tissue specimens

Human nasal polyps located in the middle meatus were surgically removed from the patients with CRS. Control samples were obtained from removed normal mucosal membranes of sphenoid sinus at operation of pituitary adenoma. The samples were fixed in 10% formalin, embedded in paraffin, processed routinely and stained with hematoxylin-eosin.

Immunohistochemistry

The nasal polyps were fixed in 10% formalin, embedded in paraffin, processed routinely and then prepared as routine semithin sections (3.5 μm). The EG2 antibody was purchased from Pharmacia (Uppsala, Sweden). The sections were stained by the Ventana iVEWTM DAB Detection kit using a Ventana automated stainer (Ventana Japan K.K., Yokohama, Japan). Sections treated with control mouse IgG1 served as negative controls.

Analysis of infiltrated eosinophils into the epithelium and subepithelium

To evaluate the degree of eosinophilic infiltration, two of the authors independently counted the number of eosinophils in 3 fields with cell clusters using light microscopy (400x magnification).

Analysis of the epithelial damage and basement membrane thickness

Epithelial damage and BM thickness was observed on the sections stained with hematoxylin-eosin. The images were acquired using a CCD camera connected to a personal computer. The length of the epithelia and BM thickness were measured using NIS Elements-D (Nikon, Tokyo, Japan).
The staging of epithelial damage allowed for the quantification of epithelial loss. The length of epithelial sloughing was expressed as a percentage of the total epithelial length [25]. Epithelial damage of the control group would include physiological sloughing and artifacts. In fact, it is difficult to completely omit such artifacts. Therefore, epithelial damage was observed under the same conditions in both the patients with nasal polyps and the control group. Surgeons with equivalent expertise carefully removed all samples. The samples were fixed in formalin and stained with hematoxylin-eosin with the same procedures and manners. The BM thickness was selected in the 3rd field from the top (400x magnification) located in severely thickened regions and the mean was calculated after inspection of the entire specimens. In this study, BM thickness included subepithelial fibrosis beneath the BM [17].

Statistical analyses

The data were expressed as the mean ± S.D. Statistical analyses were performed using Pearson's correlation coefficient and Student's *t*-test in StatMate III for Windows. Differences were considered to be significant if $p < 0.05$.

RESULTS

Minimal proliferation of the epithelial cells or goblet cells was observed in the epithelial layers of the control group, whereas there was slight but apparent epithelial sloughing. The subepithelial layers showed only a few inflammatory cells and fibroblasts (Figure 1A). The mean numbers of eosinophils in the epithelial and subepithelial layers were 0.1 ± 0.1 and 2.6 ± 6.1 per field, respectively. The mean percentage of epithelial damage was 15.4 ± 2.6%. The mean BM thickness was 4.7 μm ± 5.2. In patients with CRS, the subepithelial layers showed many inflammatory cells with proliferating fibroblasts and the epithelial layers contained both proliferating epithelial cells and goblet

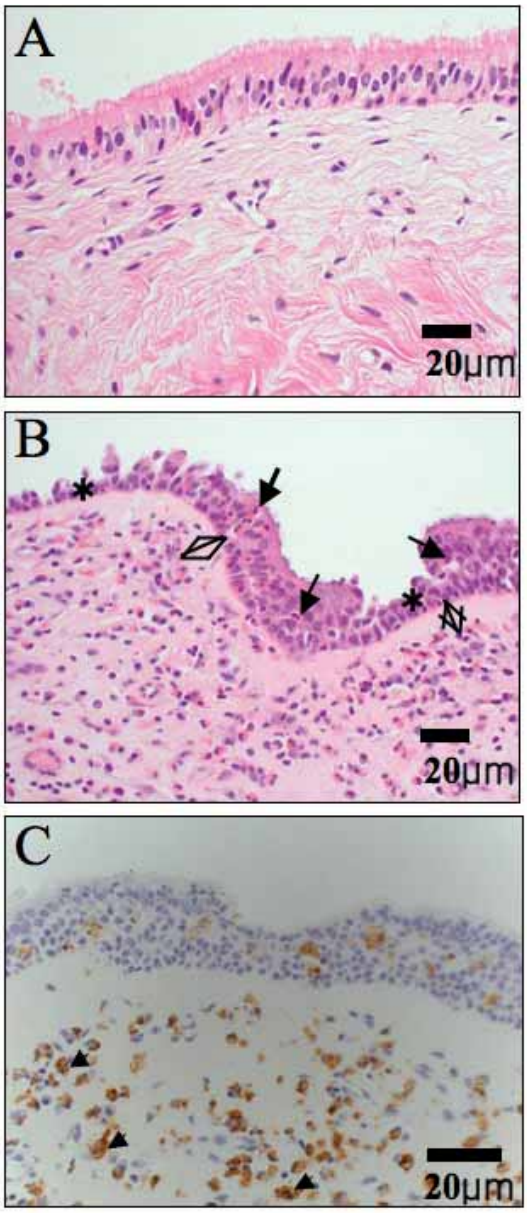

Figure 1. Histopathological sections from normal sinus mucosa and nasal polyps sections. A: normal sinus mucosa of the control group shows little or no epithelial sloughing, eosinophils and BM thickening. B: nasal polyp sections from the CRS group shows aggressive infiltrating eosinophils (arrows), severe epithelial sloughing (asterisks) and BM thickness (double arrows). C: Most of the EG2 positive cells in the nasal polyps are eosinophils. Some of the EG2 positive cells are self-melted and engulfed by macrophages (arrows).

cells with infiltration of some eosinophils (Figure 1B). The mean numbers of eosinophils in the epithelial and subepithelial layers were 3.0 ± 3.7 and 122.0 ± 173.2 per field, respectively. Among the eosinophils identified by hematoxylin-eosin staining, 90.7 ± 5.0% (n = 5) showed EG2 positive activated type (Figure 1C). Eosinophils in CRS patients were significantly greater than those in the control group (epithelial layers, $p < 0.001$; subepithelial layers, $p < 0.001$). The average epithelial damage and BM thickness in CRS patients were 62.1 ± 23.3%

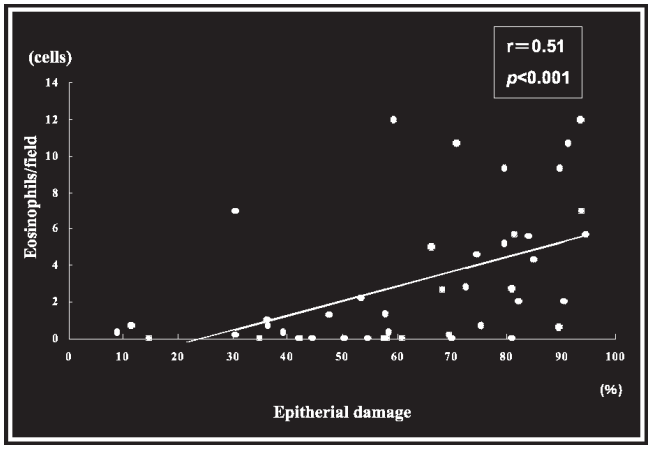

Figure 2. Relationship between epithelial damage and eosinophilic infiltration into the epithelia.

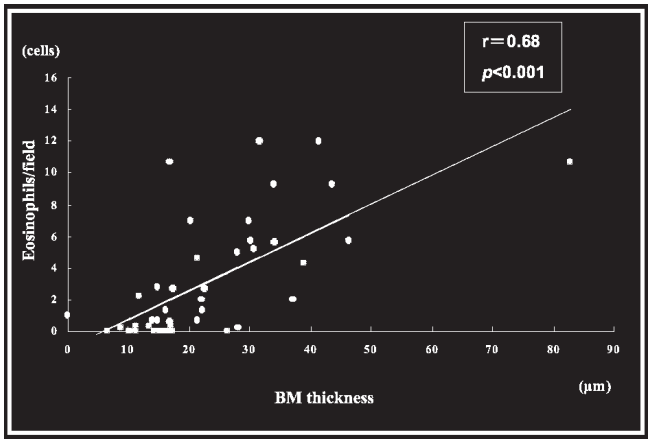

Figure 3. Relationship between BM thickness and eosinophilic infiltration into the epithelia.

and 22.6 μm ± 13.9, respectively, both of which were significantly greater than those in the control group ($p < 0.001$).

The patients were divided into asthma (n = 18) and non-asthma (n = 27) groups. Epithelial damage in the asthma and non-asthma groups was 68.6 ± 19.5% and 57.8 ± 24.9%, respectively. Similarly, BM thickness was 21.8 μm ± 11.6 and 23.1 μm ± 15.4, respectively, in these groups. Neither parameter showed significant differences between the asthma and non-asthma groups.

The eosinophilic infiltration in the epithelial layer showed a significant correlation with the epithelial damage ($r = 0.51$, $p < 0.001$, Figure 2) and BM thickness ($r = 0.68$, $p < 0.001$, Figure 3). The eosinophilic infiltration in the subepithelial layer also had a significant correlation with the epithelial damage ($r = 0.47$, $p < 0.01$, Figure 4) and BM thickness ($r = 0.43$, $p < 0.01$, Figure 5). The correlation coefficient ($r = 0.51$) of the epithelial eosinophils to epithelial damage was larger than that ($r = 0.47$) of the subepithelial eosinophils. Similarly, the correlation coefficient ($r = 0.68$) between epithelial eosinophils and BM thickness was larger than that ($r = 0.43$) of the subepithelial

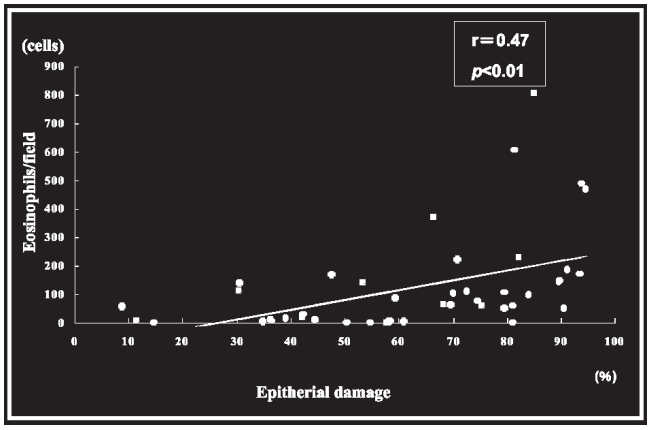

Figure 4. Relationship between epithelial damage and eosinophilic infiltration into the subepithelia.

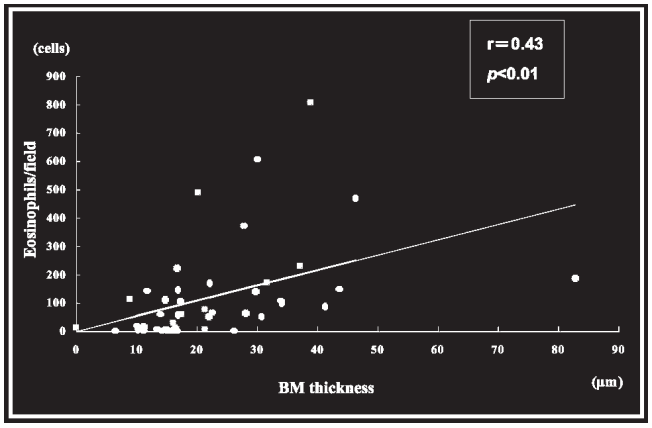

Figure 5. Relationship between BM thickness and eosinophilic infiltration into the subepithelia.

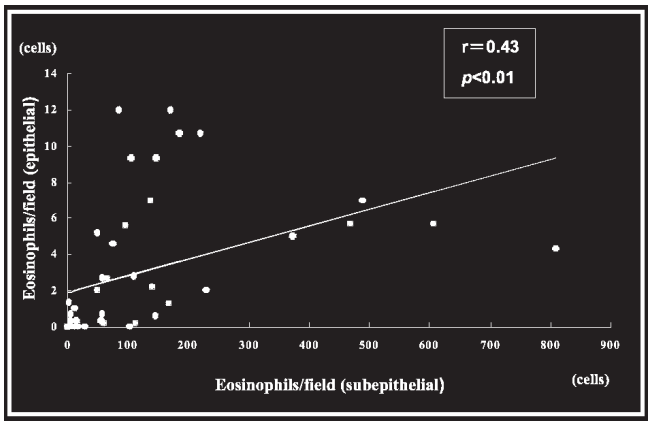

Figure 6. Relationship between the epithelial or subepithelial layers and eosinophilic infiltration.

eosinophils. The eosinophilic infiltration showed a significant correlation between the epithelial and subepithelial layers ($r = 0.43$, $p < 0.01$, Figure 6).

DISCUSSION

Ponikau et al. [20] showed eosinophilic infiltration, epithelial damage and BM thickening in the sinonasal specimens obtained from patients with CRS. However, they did not examine the relationship between eosinophilic infiltration and either epithelial damage or BM thickness. The present study is the first report to demonstrate a relationship between epithelial damage or BM thickness and eosinophilic infiltration into both the epithelial and subepithelial layers associated with the nasal polyps of CRS.

A significant correlation was noted between epithelial sloughing and eosinophilic infiltration into both the epithelial and subepithelial layers. Thomas et al. [28] suggested that the persistent and predominant infiltration of eosinophils was a histological hallmark of CRS. Wei et al. [27] examined the chemotactic behavior of eosinophils in patients with CRS. They showed that patients with CRS and all healthy control subjects demonstrated a concentration-dependent increased migration of eosinophils in the presence of both nasal mucin and tissue extracts. The percentage of migration was consistently higher for eosinophils from patients with CRS in comparison to those from the control subjects. Hamilos et al. [12] reported that cytokine patterns in sinus tissue of CRS are highly similar to bronchial tissue in asthma patients, as indicated by the presence of eosinophils under both conditions. Gleich et al. [30] suggested that releasing granule proteins from eosinophils may cause damage to the surrounding tissues. Eosinophils, which have some cytotoxic mediators, such as eosinophilic peroxidase [31], are thought to cause severe damage to the epithelia of the nasal polyps observed in the present study. Moreover, the present findings showed a significant correlation of eosinophilic infiltration between the epithelial and subepithelial layers and suggested a chemotactic gradient from the vessels to the sinus lumen across the mucosa. Therefore, it is likely that migrating and prolonging eosinophils into the epithelia continue secreting cytotoxic mediators and finally directly aggravate epithelial damage. Furthermore, the correlation coefficient ($r = 0.51$) between eosinophilic infiltration in the epithelia and epithelial damage was higher than that ($r = 0.47$) between eosinophilic infiltration in the subepithelia and epithelial damage, thus suggesting that epithelial eosinophils may contribute much more to epithelial damage than the subepithelial events.

The present study showed a significant correlation between BM thickness and eosinophilic infiltration into both the epithelial and subepithelial layers associated with nasal polyps of CRS. Ponikau et al. [20] reported eosinophilic infiltration and features of airway remodeling like epithelial sloughing and thickening of the BM in both CRS and asthma patients. Nonaka et al. [32] also claimed that eosinophilic infiltration is associated with structural abnormalities such as fibrosis and thickening of the BM. The present study supports the prediction that a central feature of an inflammatory process including BM thickening is the prevalence of eosinophils. Furthermore, the correlation coefficient ($r = 0.68$) between eosinophilic infil-

tration in the epithelia and BM thickness was higher than that ($r = 0.43$) between eosinophilic infiltration in the subepithelia and BM thickness, thus suggesting that epithelial eosinophils contribute much more to the BM thickness than the subepithelial events, such as epithelial damage.

CONCLUSION

This study examined the relationship between epithelial damage and infiltration of eosinophils in patients with nasal polyps of CRS. Epithelial damage was graded according to the degree of epithelial detachment or denudation. The results of this study showed aggressive infiltration of eosinophils into both the epithelial and subepithelial layers found along with severe epithelial damage and BM thickness, thus suggesting that eosinophilic infiltration in the epithelial layer could play a role in the process of mucosal remodeling of CRS with nasal polyps.

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