clarify the immunological characteristics. The present study proposes a new subclassification of CRSwNP, defined by eosinophilia and neutrophilia in the sinonasal tissue and secretion.

MATERIALS AND METHODS

Patients

The patients with CRSwNP, visiting the Department of Otorhinolaryngology, Juntendo University Hospital, Tokyo, Japan, and undergoing endoscopic sinus surgery between January 2008 and December 2010, were consecutively recruited after giving informed consent; the clinical profiles were analyzed, and the H & E samples of the nasal polyps were examined. The study was approved by the ethics committee of the Juntendo University Faculty of Medicine.

CRSwNP was diagnosed based on the criteria of the European position article.[2] The presence of nasal discharge, postnasal drainage, nasal obstruction, headache, and anosmia was recorded and the severity was defined as follows: severe = 3, moderate = 2, slight = 1, and absent = 0—based on a previous article.[11] The disease extent on computed tomographic (CT) scans was categorized according to Lund and Mackay.[12] None of the patients were treated with antibiotics, systemic or topical corticosteroids, or other immune-modulating drugs for at least 1 month before the surgery. Allergic fungal rhinosinusitis was excluded by the absence of specific IgE antibodies against fungi, and the absence of fungi in the sinus effusion using cytological staining and microbiological examination in the present subjects. Aspirin intolerance was defined as the exacerbation of nasal symptoms triggered by aspirin or NSAID exposure. The diagnosis was confirmed in some patients by oral challenge with aspirin. Polyp recurrence defined as the presence of nasal polyps in the middle meatus, detected by nasal endoscopy during postoperative follow-up, ranged from 15 to 48 months (28 ± 12 months).

Immunohistochemistry

Surgically removed nasal polyps located in the middle meatus with CRSwNP were randomly selected and examined for immunohistochemistry. Ten subjects from whom normal mucosal membranes of the sphenoid sinus were removed during surgery for pituitary adenoma were used as controls. The samples were fixed in 10% formalin, embedded in paraffin wax, processed routinely, and then prepared as routine semithin sections (3.5 μm). Immunohistochemistry was performed, as in our previous reports.[6,7] Briefly, the sections were treated with 0.3% H_2O_2 to quench endogenous peroxidase and blocked with normal serum after deparaffinization. The sections were treated with each primary antibody containing 1% bovine serum, and treated with a cocktail of HQ-labeled antibodies (HQ is a proprietary hapten covalently attached to the goat antibodies) and a mouse monoclonal antiHQ-labeled horseradish peroxidase tertiary antibody. The color was developed by 3, 3′-diaminobenzidine tetrahydrochloride chromogen. The primary antibodies were mouse antihuman major basic protein (MBP) (Abcam, Tokyo, Japan) 1:3, mouse antihuman neutrophil elastase (Dako, Tokyo, Japan) 1:100, rabbit antihuman eotaxin (Abcam) 1:30, rabbit antihuman IL-8 (abcam, Japan) 1:250, rabbit antihuman IL-17A (Santa Cruz Biotechnology, Santa Cruz, CA) 1:80, mouse antihuman CD68 1:3, rabbit antihuman Cu/Zn superperoxide dismutase (SOD) (Stressgen Bioreagents Corp,) 1:200, mouse antihuman MUC5AC (BIO SCIENCE FOR THE WORLD, Santa Barbara, CA) 1:3. The sections were stained by the Ventana iVEWTM DAB Detection kit using a Ventana automated stainer (Ventana Japan K.K., Yokohama, Japan). The sections treated with control mouse and rabbit IgG1 served as negative controls.

Quantification of Eosinophils and Neutrophils in Tissues

To evaluate the degree of cell infiltration, two of the authors (N.O., T.K.) independently counted the number of eosinophils and neutrophils determined by H & E stains in the three fields containing the greatest degree of cellular infiltration using light microscopy ($\times$ 400 magnification). The total number of eosinophils and neutrophils present with a 10 $\times$ 10-mm reticulate present in the eyepiece was determined as the count per high-power field (HPF). Eosinophilic mucin was defined as thick, strongly colored mucus in the sinuses, confirmed at surgery and recognized as the presence of eosinophil or eosinophil-degraded products in mucus by histology.

Statistics

The data were expressed as the mean $\pm$ S.D. Statistical analyses were made using StatMate IV for Windows (Tokyo, Japan). Data of three-group comparison were evaluated using analysis of variance for age; Pearson's chi-square for the gender and the recurrence rates; and Kruskal-Wallis one-way analysis of variance for serum eosinophils, IgE, symptomatic scores, and CT scores. A two-sample t test was used for a two-group comparison of age. Pearson's chi-square test was used for a two-group comparison of the gender and the recurrence rates. An one-way analysis of variance was followed by a Mann-Whitney test for a two-group comparison of serum eosinophils, IgE, symptomatic scores, and CT scores. The significance of differences among the three groups in immunohistochemical parameters was analyzed by one-way analysis of variance. Differences were considered to be significant if $P < 0.05$.

To derive cutoff values of eosinophils and neutrophils in order to define as eosinophilic and neutrophilic CRS, respectively, we divided patients in the derivation set into 10 cells/HPF. We constructed receiver operating characteristics curves of the number of patients with polyp recurrence for the number of eosinophils and neutrophils to find the best cutoff value, and fitted Cox proportional hazard model to obtain the hazard ratio and 95% confidence intervals—representing the increase in cutoff value per 10 cells. The statistical analysis was performed using a Chi-square test.

RESULTS

Subclassification of Chronic Rhinosinusitis With Nasal Polyps

One hundred thirty patients with CRSwNP (36 females and 94 males, ranging in age from 13 to 80 years, mean age 54 ± 13 years) were enrolled in this study. The eosinophilic and neutrophilic counts associated with polyp recurrence are shown in Figure 1. The patients between 90 and 110 eosinophils/HPF showed the minimum P values ($P = 0.005$). The patients with $\geq$20 neutrophils/HPF exhibited the minimum P values ($P = 0.0026$). Therefore, eosinophilic and neutrophilic polyps were defined as eosinophil counts of more than 100 cells/HPF and neutrophil counts of more than 20 cells/HPF, respectively. Noneosinophilic nonneutrophilic polyps were defined as those with both eosinophil counts of less than 100 cells/HPF and neutrophil counts of less

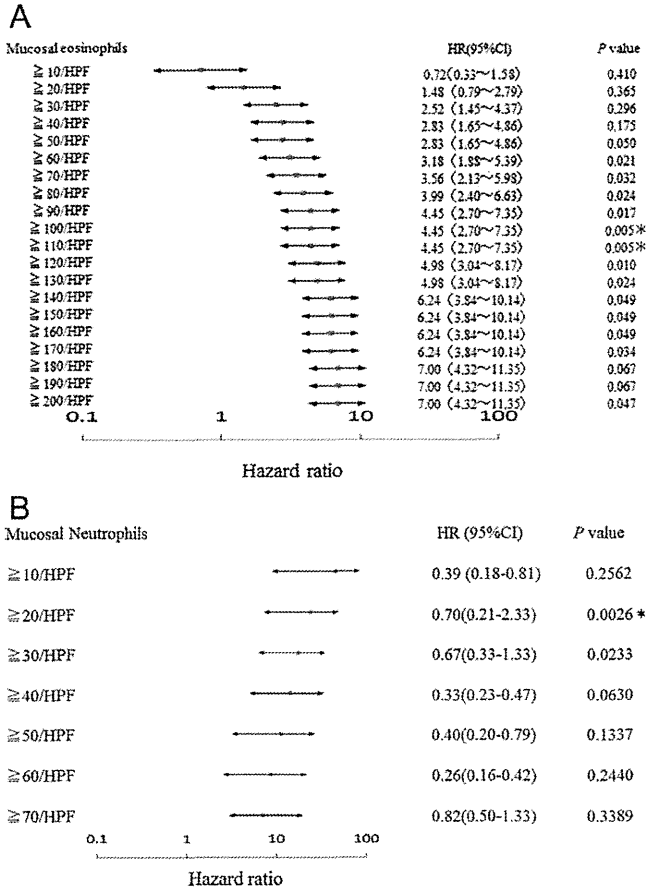

Fig. 1. Association of mucosal eosinophils (A) and neutrophils (B) with polyp recurrence. To derive cutoff values of eosinophils and neutrophils in order to define as eosinophilic and neutrophilic CRS, respectively, the patients were divided in the derivation set into 10 cells/high-power field (HPF). We constructed receiver operating-characteristics curves of the number of patients with polyp recurrence for the number of eosinophils and neutrophils to find the best cutoff value, and fitted Cox proportional hazard model to obtain the hazard ratio (HR) and 95% confidence intervals (CI), representing the increase in cutoff value per 10 cells. The patients between 90 and 110 eosinophils/HPF showed the minimum *P* values (*). The patients with ≥ 20 neutrophils/HPF exhibited the minimum *P* values (*).

than 20 cells/HPF. On this basis, the eosinophil count with H& E findings of nasal polyps (Fig. 2A), 130 patients were first classified into 42 patients with eosinophilic type and 88 patients with noneosinophilic type. Next, 88 noneosinophilic type was divided into 27 patients with neutrophilic type and 61 patients with noneosinophilic and nonneutrophilic type, based on the neutrophil count. Furthermore, the activated types of eosinophils and neutrophils were determined by staining with MBP and neutrophil elastase, respectively. Eosinophils positive for MBP were significantly higher in the eosinophilic group than in the other two groups, both of which showed marked reductions of infiltrated eosinophils (Figs. 2B and 2D). Although the neutrophilic group showed increased numbers of neutrophil elastase-positive cells compared to the other two groups, moderate numbers of neutrophils infiltrated into the nasal polyps were recognized in the eosinophilic group (Figs. 2C and 2D).

Clinical Profiles

Among the three subgroups, there was no significant difference in age. The incidence of males was significantly higher than that of females in the noneosinophilic nonneutrophilic group compared to the other two groups. Both the numbers of serum eosinophils and the recurrence rates were significantly higher in the eosinophilic group compared to the other two groups. The IgE value was significantly higher in the eosinophilic group, followed by the noneosinophilic nonneutrophilic and neutrophilic groups. Both the symptomatic and CT scores were significantly higher in the eosinophilic group than in the neutrophilic group (Fig. 3).

Next, eosinophilic CRSwNP (n = 42) was divided by the presence or absence of aspirin-induced exacerbation. The patients with aspirin-exacerbated eosinophilic CRSwNP (n = 11) significantly differed from those without evidence of aspirin sensitivity (n = 31) in gender, but not in the other parameters (Fig. 4). Finally, eosinophilic CRSwNP patients who evidenced no episodes of aspirin-induced exacerbation (n = 31) were classified according to the presence (n = 10) or absence (n = 21) of eosinophilic mucin in the sinus. The number of serum eosinophils, IgE values, and CT scores were significantly higher in mucin eosinophilic CRSwNP than in the nonmucin group. There were no differences in age, gender, symptomatic scores, or recurrence rates between mucin and nonmucin eosinophilic CRSwNP (Fig. 5).

Immunohistochimical Analysis

The immunopathologic features obtained from 42 patients (17 females and 25 males, ranging in age from 21 to 75 years; mean age 53 ± 13 years) of eosinophilic (n = 30), neutrophilic (n = 6), and noneosinophilic nonneutrophilic (n = 6) subgroups of CRSwNP mentioned above were evaluated according to the expression of eotaxin, IL-8, IL-17A, CD68, Cu/Zn SOD, and MUC5AC in the nasal polyp tissues (Fig. 6). The expressions of eotaxin, IL-17A, MUC5AC, and CD68 were upregulated in the eosinophilic group compared to the other two groups. IL-8 expression was significantly decreased in the noneosinophilic nonneutrophilic group compared to the other two groups. Cu/Zn SOD-positive cells were significantly reduced in the eosinophilic group compared to the other two groups (Fig. 7). The aspirin-exacerbated type showed a significant increase in the infiltrated cells positive for IL-8, IL-17A, and MUC5AC compared to the aspirin-tolerant type. Significantly different expression between the mucin and nonmucin types was observed only in IL-17A.

DISCUSSION

A general grouping of CRS into forms with nasal polyps and those without nasal polyps has been accepted.[1,2] CRSwNP in Caucasian populations is

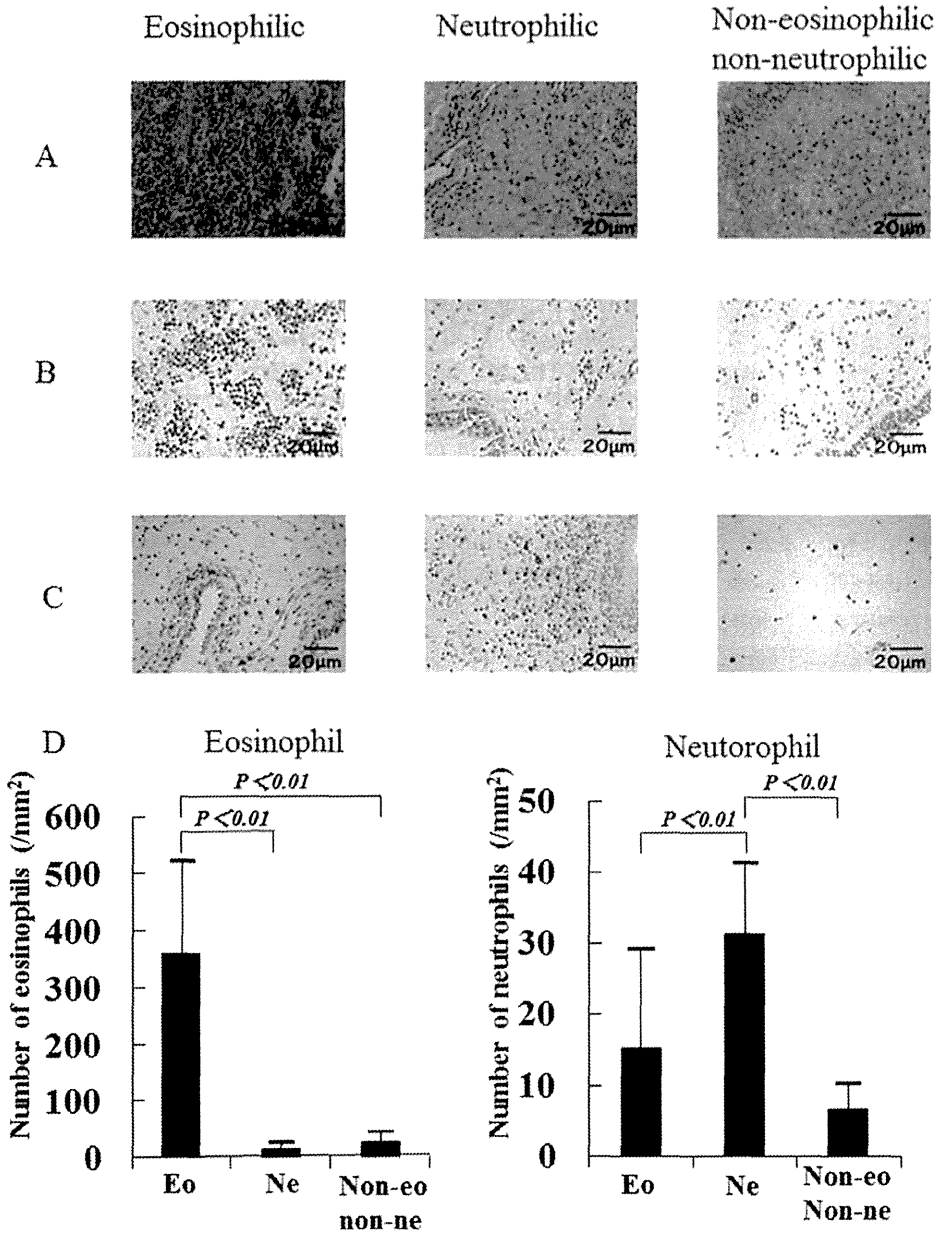

Fig. 2. Subclassification of chronic rhinosinusitis with nasal polyps. (A) H & E histopathology; (B) immunohistochemical staining with major basic protein; and (C) neutrophil elastase in nasal polyps of eosinophilic, neutrophilic, and noneosinophilic nonneutrophilic types were presented. (D) The numbers of activated eosinophils and neutrophils infiltrated in nasal polyps were evaluated in eosinophilic (Eo, n = 30), neutrophilic (Ne, n = 6), and noneosinophilic nonneutrophilic (Non-eo non-ne, n = 6) groups.

believed to be enriched in tissue eosinophils and related biomarkers.[13,14] However, stronger arguments that eosinophils are not the end-all of the CRSwNP pathogenesis have been made. The nasal polyps from southern China have been suggested to poorly express eosinophilia.[10] Based on our previous study,[9] two positive feedback mechanisms of neutrophil accumulation involving IL-1β and IL-8 in the sinus tissues and cavities are applied to both CRS with and without nasal polyps in the Japanese population. Thus, the CRSwNP phenotype forms heterogenous groups, at least in Asian populations.

The asthma phenotype is postulated to be both clinically and pathologically heterogenous. Four subtypes have been identified: eosinophilic, neutrophilic, paucigranulocytic, or mixed cellularity—depending on the presence or absence of sputum eosinophils and/or neutrophils.[15] The inflammatory phenotype of CRSwNP in the present study was subclassified into eosinophilic, neutrophilic, and noneosinophilic nonneutrophilic types, according to the presence or absence of eosinophils and/or neutrophils in the tissues.

The definition of mucosal eosinophilia in CRSwNP is still controversial. Soler et al.[16] used a cut-point of >5 eosinophils/HPF to define clinically relevant mucosal eosinophilia based on in vivo evidence of eosinophil activation.[17] More currently, [18] they observed the impact of eosinophilia on quality-of-life outcome above 10 eosinophils/HPF. On the other hand, the molecular and biological nature of nasal polyps in the Japanese population seems to be different than those of Western countries. Matsuwaki et al.[19] documented that <120 eosinophils/HPF was most strongly correlated with the recurrence of CRS. Moreover, from the same group of the study, the patients with >70 eosinophils/HPF had the highest recurrence rate of nasal polyps.[20] Therefore, the present

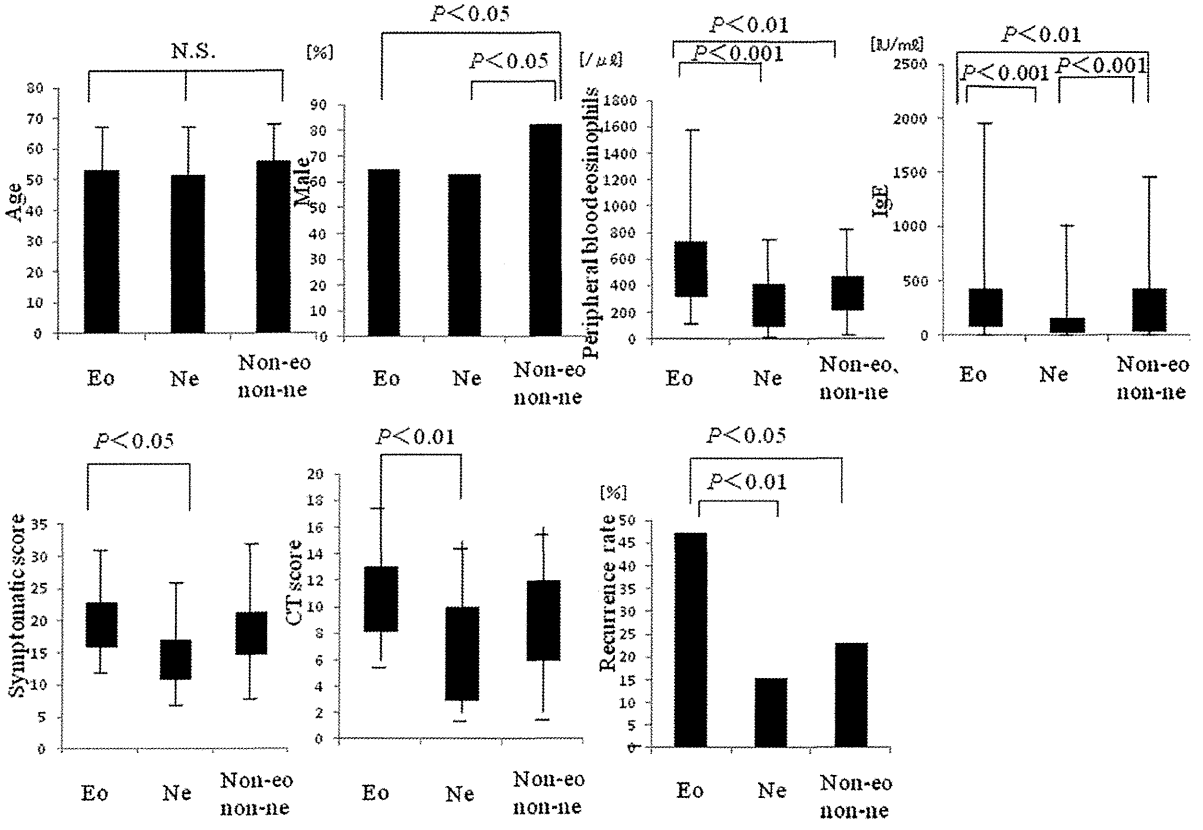

Fig. 3. Comparison of the clinical profiles among eosinophilic (Eo), neutrophilic (Ne), and noneosinophilic nonneutrophilic (Non-eo non-ne) groups of chronic rhinosinusitis with nasal polyps.

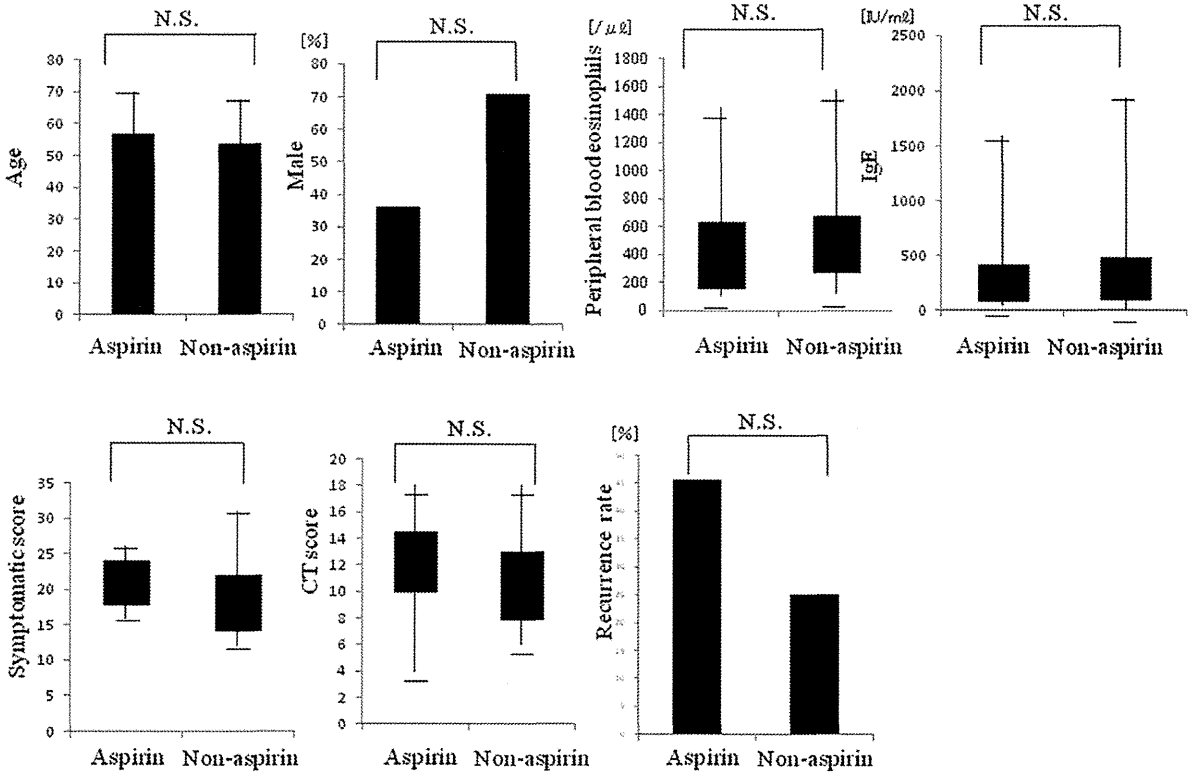

Fig. 4. Comparison of the clinical profiles between aspirin-sensitive (Aspirin) and aspirin-insensitive (Non-aspirin) subgroups of eosinophilic chronic rhinosinusitis with nasal polyps.

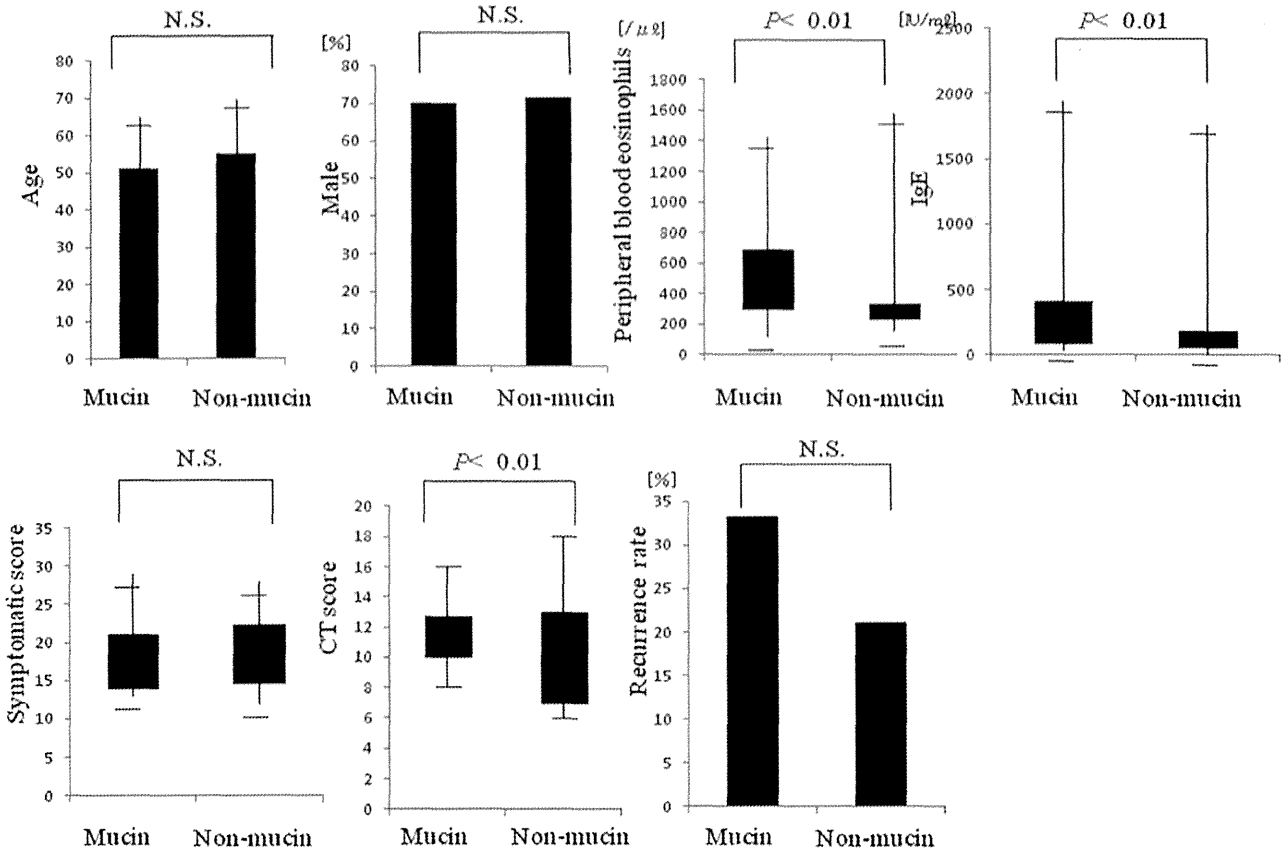

Fig. 5. Comparison of the clinical profiles between mucin and nonmucin subgroups of eosinophilic chronic rhinosinusitis with nasal polyps.

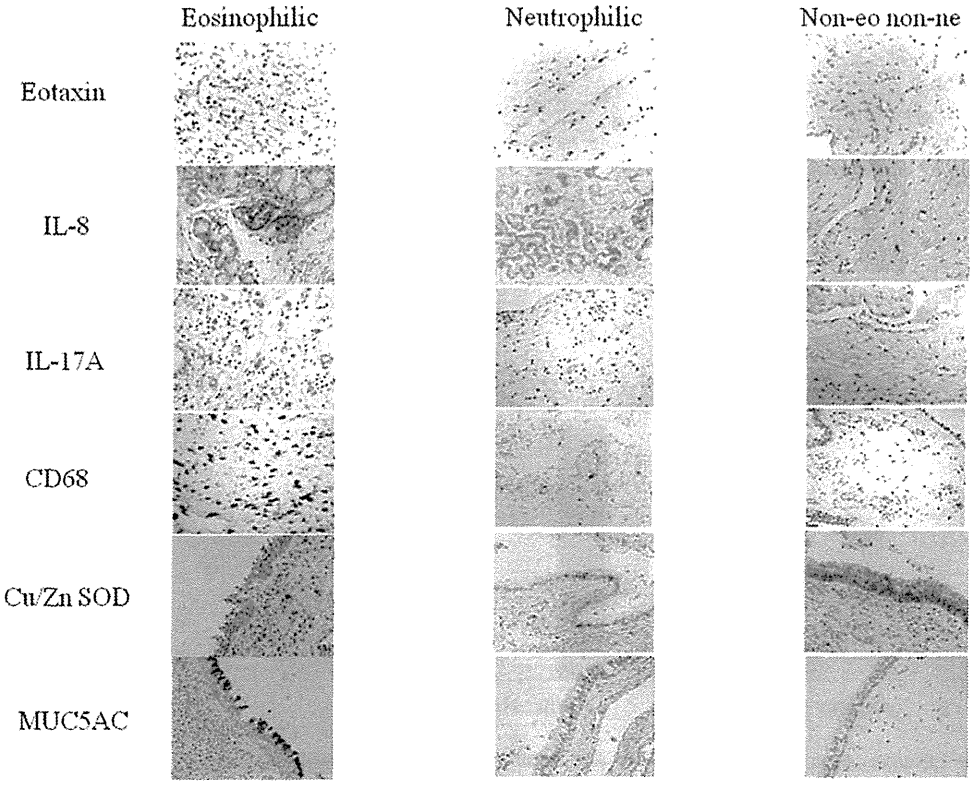

Fig. 6. Immunohistochemistry of eotaxin, IL-8, IL-17A, CD68, Cu/Zn superperoxide dismutase (SOD), MUC5AC in eosinophilic, neutrophilic, and noneosinophilic nonneutrophilic (Non-eo non-ne) groups of chronic rhinosinusitis with nasal polyps.

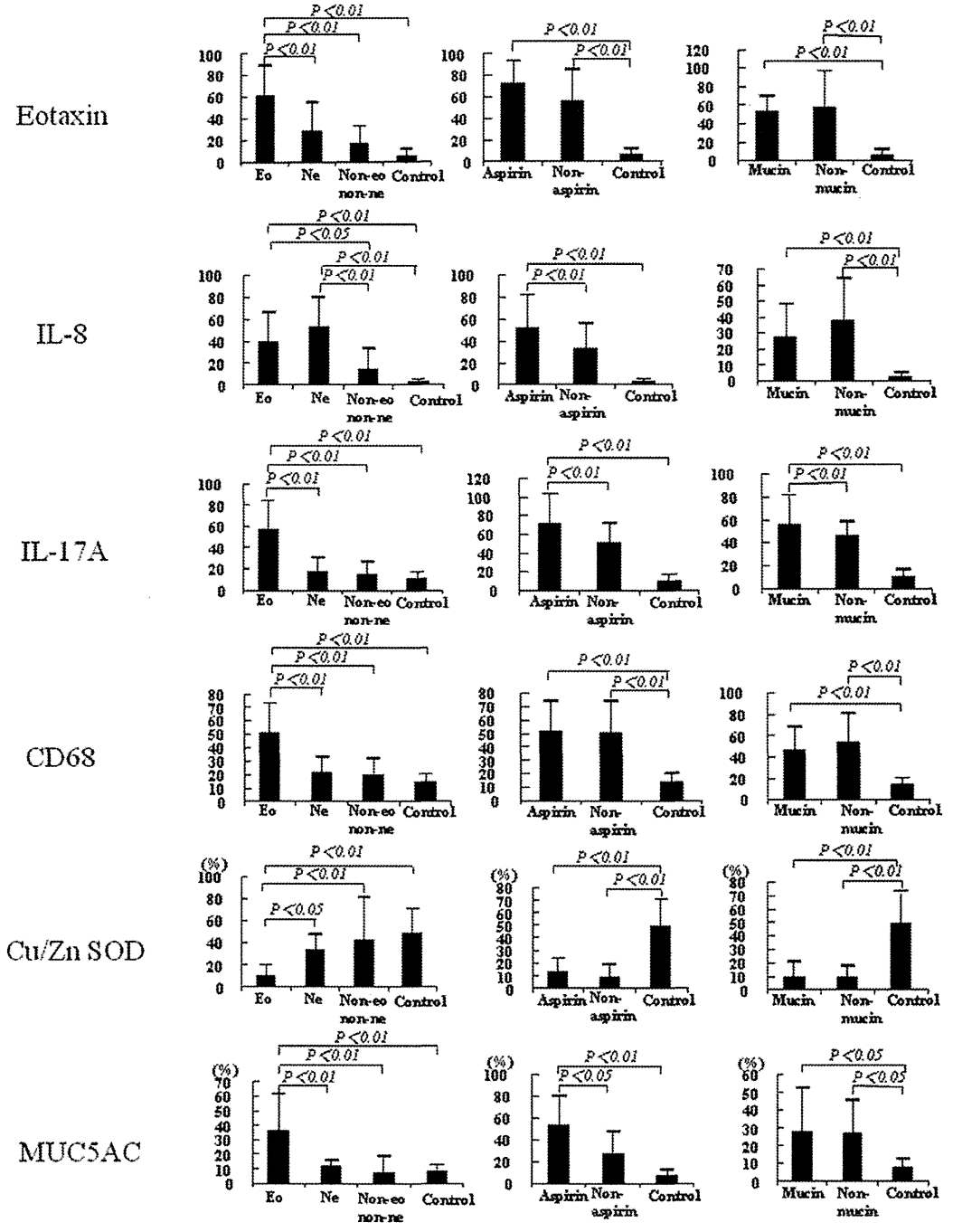

Fig. 7. Comparison of the number of the infiltrated cells positive for eotaxin, IL-8, IL-17A, and CD68; and the percentage of epithelial cells positive for Cu/Zn superperoxide dismutase (SOD), MUC5AC in eosinophilic (Eo, n = 30), neutrophilic (Ne, n = 6), and noneosinophilic non-neutrophilic (Non-eo non-ne, n = 6) groups of chronic rhinosinusitis with nasal polyps; and aspirin-sensitive (Aspirin) and aspirin-insensitive (Non-aspirin), mucin, and nonmucin subgroups of eosinophilic chronic rhinosinusitis with nasal polyps; and controls.

results of cutoff values of eosinophils/HPF are very consistent with the previous studies in the Japanese population.

Tissue eosinophilia in CRS is thought to be related to more extension disease and a decreased likelihood of surgical success.[21–23] Serum eosinophilia had a worse prognosis and extensive sinus disease.[21,24,25] In the present study, eosinophilic CRSwNP was characterized by serum eosinophilia, atopy, extensive disease, and poor prognosis compared to the other two subgroups. A significant difference between neutrophilic and noneosinophilic nonneutrophilic subgroups was recognized only in gender and total IgE values. Thus, the eosinophilic type can be clearly distinguished from noneosinophilic types on the basis of the clinical profiles.

Revision surgery rates and recurrence rates following endoscopic sinus surgery are higher in patients with aspirin intolerance compared to those with aspirin

tolerance.[26–28] However, that no significant difference in the recurrence rates between aspirin tolerance and intolerance was found in the present study may be due to the shortness of the postoperative follow-up periods.[28] Although the clinical profiles showed no significant differences between the aspirin-intolerant and tolerant types, immunohistochemical parameters such as IL-8, IL-17A, and MAC5AC were significantly upregulated in the aspirin-intolerant type, suggesting severe inflammation. The disease with eosinophilic mucin is postulated to be broadly divided into nonfungal and fungal categories.[29] The present study excluded CRSwNP related to fungus based on the negative fungal-specific IgE and the absence of fungal hyphae examined by histopathology and bacteriology of the mucous. Serum eosinophilia, atopy, and disease severity, based on the CT scores demonstrated in CRSwNP in the presence of eosinophilic mucin, would be the characteristic clinical feature of mucin CRSwNP.

Eotaxin and IL-8 exhibit potent and selective chemoattractive activities for eosinophils and neutrophils, respectively. Eosinophil-dominant polyps of CRSwNP are reported to show significant expression of eotaxin compared to noneosinophilic CRSwNP,[6] which was also confirmed by the present study. Eotaxins (eotaxin-1, 2, 3) have a preference in the activation of eosinophils via a single C-C chemokine receptor, the CCR-3. Thus, eotaxin and selective CCR-3 receptors differentiate the therapeutic approach. IL-8 expression is highly observed in the sinonasal mucosa in CRS with neutrophil-rich secretion.[8] One of the target molecules of macrolide therapy for CRS is IL-8, which is produced in neutrophils, eosinophils, and epithelial cells.[9] The present findings that IL-8 was highly expressed in eosinophilic CRSwNP, as well as in the neutrophilic group, may support the idea that macrolide therapy is also effective in the eosinophilic-dominant pathology of CRSwNP.[30] These two chemokines are secreted in nasal discharge, which may represent potential candidates of diagnostic biomarkers to distinguish between eosinophilic and neutrophilic CRSwNP.

The increased expression of IL-17A in asthmatic lungs is proposed to induce the increased accumulation and activation of lung neutrophils and neutrophil-activating cytokines.[31] However, the localization of IL-17A expression predominantly coincided with eosinophils and CD4-positive lymphocytes in Japanese CRSwNP. The infiltration of the cells both positive for CD4 and IL-17A (Th17 cells) showed a significant correlation with the number of eosinophils and mucosal remodeling in Japanese populations.[7,32] The present study showed that eosinophilic CRSwNP had a moderate number of neutrophils (Fig. 2), indicating the involvement of neutrophilic inflammation. Neutrophils are recruited by IL-8, which is mediated by IL-17A in asthmatic airways.[33] Taken together, Th17 cells may play a key role in regulating the recruitment of both eosinophils and neutrophils in eosinophilic CRSwNP, at least of Asian populations. Furthermore, the significant upregulation of IL-17A expression in aspirin-exacerbated and mucin types supports the idea that the eosinophil-dominant pathway of Asian nasal polyps is driven by Th17 cells.

In our results, the number of macrophages with CD 68-positive reactions was higher in the eosinophilic type than in the other two types. Macrophages, as well as eosinophils, are known to produce cytotoxic agents such as oxidants and metalloproteinases.[34] The mucosal remodeling in eosinophilic CRSwNP may be associated with the infiltration of macrophages as well as eosinophils. On the other hand, there is a cytoprotective mechanism against oxidative stress. We previously demonstrated that heme-oxidase-1—an enzyme-catabolizing heme to produce monoxide, free iron, and biliverdin—showed reduced expression in eosinophilic CRSwNP.[35] Another endogenous antioxidant enzyme, Cu/Zn SOD, which was shown to be downregulated in eosinophilic CRSwNP in the present study, may result from consumption of the enzyme by neutralizing reactive oxidative species produced by eosinophils and macrophages.

Mucus hypersecretion and persistent airway inflammation result from increased expression of the MUC5AC mucin gene,[36] which is induced by IL-17A.[37] The upregulation of MUC5AC expression in eosinophilic CRSwNP implies the underlying mechanism of mucus hypersecretion.

CONCLUSION

The eosinophilic CRSwNP phenotype is clinically characterized by serum eosinophilia, atopy, extensive disease, and poor prognosis compared to the neutrophilic and the noneosinophilic nonneutrophilic groups. We clearly demonstrated that all three subgroups of CRSwNP had characteristic differences in those inflammatory markers, which allows for pathophysiologically meaningful differentiations with likely therapeutic consequences.

ACKNOWLEDGEMENTS

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論　　説

慢性鼻副鼻腔炎・鼻茸の病態と治療

池　田　勝　久

Pathophysiology and Treatment Strategy of Chronic Rhinosinusitis with Nasal Polyps

Katsuhisa Ikeda

(Juntendo University Graduate School of Medicine)

Although chronic rhinosinusitis (CRS) is a multifactorial disease in a heterogenous group of diseases with different underlying etiologies and pathophysiologies, European and US studies have proposed its classification into four categories, i) acute bacterial rhinosinusitis, ii) CRS without nasal polyps, iii) CRS with nasal polyps (CRSwNP), and iv) allergic fungal rhinosinusitis. The histomorphological patterns of CRSwNP are characterized by Th2-driven immune responses including the predominance of eosinophils and mixed mononuclear cells with a relative paucity of neutrophils. Differing from European and US patients, Japanese patients with CRSwNP are thought to be subdivided into eosinophil-dominant, neutrophil-dominant, and eosinophil- and neutrophil-paucity types. The subclassified categories of CRSwNP were evaluated and supported by the clinical backgrounds such as disease severity, atopic status, recurrence, etc. Furthermore, the expression patterns of inflammatory parameters in each group were compared in order to clarify the immunological characteristics.

The treatment strategy for eosinophilic CRS is as follows. In selective patients showing extensive and massive sinonasal pathology a 7-day course of oral predonisolone tablets was administered before and/or after endoscopic sinus surgery (ESS). A short-term (3 to 5 days) of oral predonisolone was administered when olfaction judged by the self smell test was aggravated. Moreover, antibiotics were orally given in the presence of massive purulent nasal discharge. Bacterial infection may play a critical role in recurrent polyps and refractory symptoms during post-ESS follow-up. Moreover, worsening of sinusitis accompanies asthma exacerbation.

Keywords : pathophysiology, treatment, chronic rhinosinusitis, nasal polyp, classification

序　論

国際的なコンセンサスのある鼻副鼻腔炎の分類は，①ウイルス性鼻副鼻腔炎から移行して生じる急性化膿性鼻副鼻腔炎，②鼻茸を伴わない慢性鼻副鼻腔炎，③鼻茸を伴う慢性鼻副鼻腔炎，④アレルギー性真菌性鼻副鼻腔炎である[1)2)]．本稿では主に，鼻茸を伴う慢性鼻副鼻腔炎に関する病態と治療を解説する．

病　態

本邦では，アレルギー性鼻炎に合併する副鼻腔炎としてアレルギー性鼻副鼻腔炎が鼻副鼻腔炎の範疇として提唱されている．しかしながら，I 型アレルギーと慢性鼻副鼻腔炎との間には直接的な因果関係の証拠は乏しいというのが国際的な意見であり[3)]，アレルギー性鼻副鼻腔炎という呼称は避ける傾向にある．アレルギー性鼻副鼻腔炎はアレルギー性鼻炎に合併する副鼻腔炎であり，病

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因として，鼻腔粘膜の浮腫による副鼻腔換気の障害によって副鼻腔病変が生じる説とまたは抗原が直接副鼻腔に侵入して副鼻腔粘膜自体にⅠ型アレルギー反応が生じる説の2つが唱えられている．しかしながら，これまでの研究では副鼻腔病変とアレルギー性鼻炎・鼻腔形態異常との関連にはいまだコンセンサスが得られていない．両者に関連ありとする報告は，①花粉シーズンにおいてCTで副鼻腔病変を認める[4]，②抗原の鼻腔チャレンジで副鼻腔病変がCTで観察される[5]，③血中IgE値がCTでの副鼻腔粘膜肥厚と相関する[6]などである．一方，関連なしとする報告は，①花粉症患者で花粉シーズンでも副鼻腔病変は不変[7]，②SPECT・PETでアレルギー性鼻炎による副鼻腔病変は観察されない[8]，③副鼻腔形態異常は副鼻腔炎の病因を示唆する報告は少ない[9]などである．そこで，われわれはアレルギー性鼻炎患者と非アレルギー性慢性鼻炎患者において，CTによって描出した副鼻腔病変を比較検討した．両群での副鼻腔病変の出現率に有意差はなく，副鼻腔病変はアレルギー性の有無に関係なく，鼻腔粘膜の慢性炎症で生じることが示唆された．また両群において，鼻腔抵抗とostiomeatal complex（OMC）サイズは副鼻腔病変の出現に相関を認め，慢性鼻炎における副鼻腔病変は非特異的な炎症によって生じて，鼻閉やOMCの閉塞に関連することが分かった[10]．

鼻茸を伴う慢性鼻副鼻腔炎については，副鼻腔粘膜の

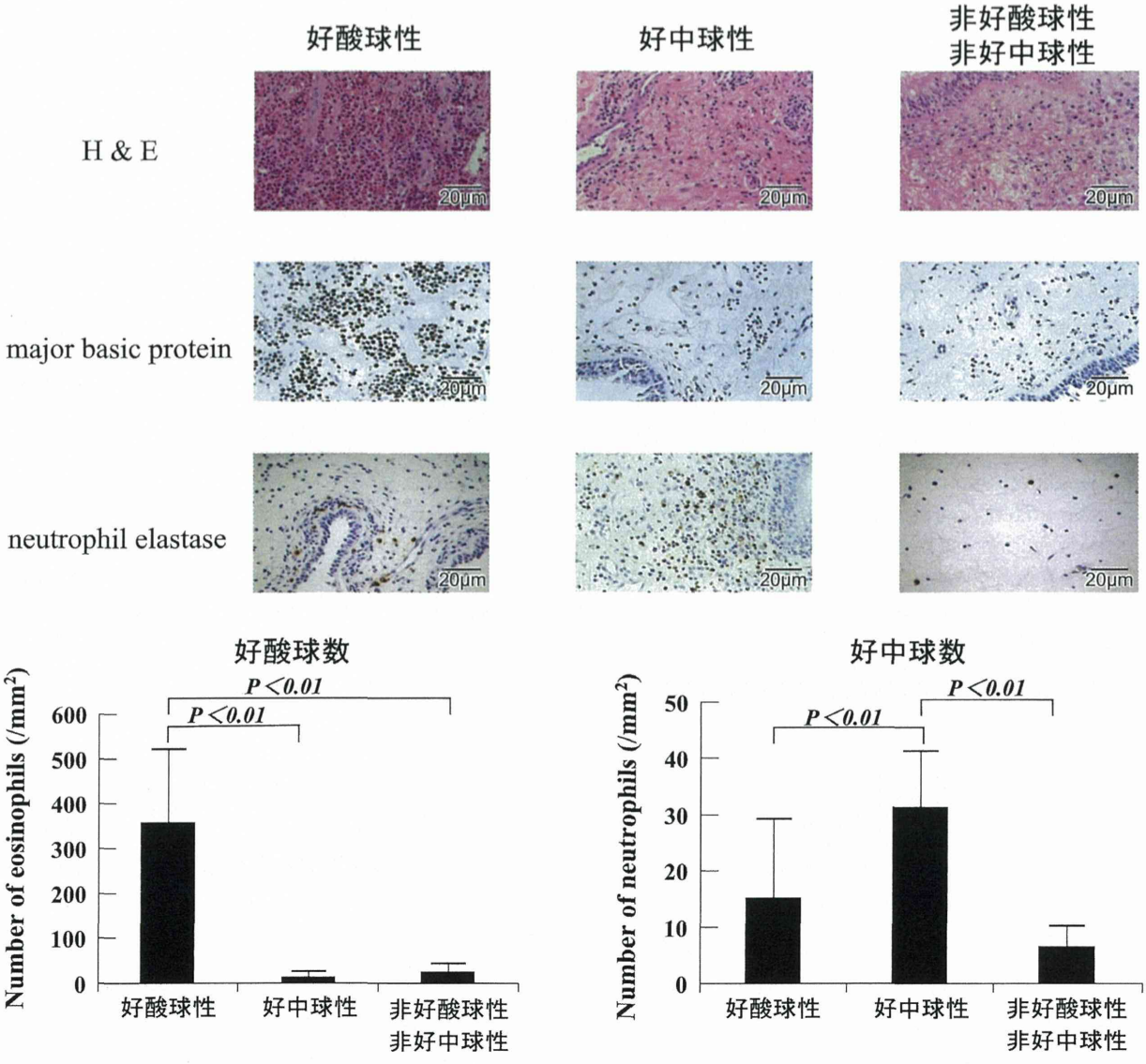

図1　好酸球性副鼻腔炎，好中球性副鼻腔炎，非好酸球性・非好中球性副鼻腔炎のH&E，major basic protein，neutrophil elastaseの染色像，鼻茸組織中の好酸球数と好中球数（文献21から改変）

病理組織学所見から好酸球型と非好酸球型に区別することで慢性鼻副鼻腔炎の亜分類を試みたことから，好酸球性副鼻腔炎の概念が生まれた[11]．1994年にNewmanら[12]は末梢血の好酸球の増多と高度な副鼻腔病変が関連することを最初に報告した．本邦では森山，春名らが好酸球性副鼻腔炎の呼称を提唱した[13][14]．その後，多くの研究で血中の好酸球数や副鼻腔の病的粘膜の好酸球浸潤の増加が病変の重症度や術後の予後不良に相関することが明らかになった[11]．

鼻茸を伴わない慢性鼻副鼻腔炎は副鼻腔の自然口の閉塞による副鼻腔からの粘液の排泄障害を起こす病態で，慢性の細菌感染やTh1反応が関係する．従来より鼻茸を伴う慢性鼻副鼻腔炎はTh2反応による好酸球性炎症が主体であると考えられてきたが，嚢胞性線維症やアジア人では好中球性炎症の関与も判明してきている[15]．中国人の鼻茸はTh1/Th17のパターンを示し，IL5の関与しないTreg系の経路が優位に作用していることが報告されている[16]．好中球炎症による慢性鼻副鼻腔炎の特徴的な病態は細菌感染などの外的因子の曝露のない状態においても，副鼻腔洞内に持続的な好中球の浸出が認められることである．この好中球動員はTh1を基盤とした免疫反応によって，ICAM-1やE-セレクチンなどの接着因子を活性化し，さらに，副鼻腔に浸出した活性化好中球はエラスターゼ，プロテアーゼなどの蛋白分解酵素や活性酸素を放出し粘液線毛機能を低下させ，病態形成の中心的役割を演じている．慢性鼻副鼻腔炎における鼻汁中への好中球の動員の機序としてIL-8の関与が次の証拠から明らかになった[17]．①鼻汁中の好中球と鼻粘膜上皮におけるIL-8の存在，②好中球と鼻粘膜におけるIL-8のmRNAの発現，③in vitroでの好中球遊走能の10倍以上のIL-8量の鼻汁中での存在，④鼻汁中のIL-8量と好中球浸出量の相関である[18][19]．マクロライドの半量長期療法[20]の作用機序の一つがIL-8分泌の抑制効果である．その結果慢性鼻副鼻腔炎の遷延化の機序であるIL-8による好中球の動員の悪循環が打ち切れるのである[17]．また鼻茸には好酸球や好中球に乏しく，少量の形質細胞やリンパ球の浸潤のみを示す症例も存在する．したがって，鼻茸を伴う慢性鼻副鼻腔炎は好酸球性副鼻腔炎，好中球性副鼻腔炎，非好酸球性・非好中球性副鼻腔炎（後2者を合わせると非好酸球性副鼻腔炎）に亜分類することができよう[21]．図1には上述の3群のH&E，major basic protein，neutrophil elastaseの染色像と3群での鼻茸の好酸球数と好中球数の比較を示している．好酸球数は好中球性副鼻腔炎，非好酸球性・非好中球性副鼻腔炎ともに好酸球性副鼻腔炎よりも低かった．また好中球数は好中球群で他の2群に比して高かったが，好酸球性副鼻腔炎においても比較的好中球を鼻茸に認めていた．臨床像の解析では，血中好酸球数と再発率で好酸球群がもっとも高かった．血清IgE値は好酸球群，非好酸球性・非好中球群，好中球群の順に高かった．症状スコアとCTスコアでは好酸球群が好中球群に比して高かった．サイトカインや炎症の関連物質を免疫組織学的に検討すると，IL-17A, MUC5AC, CD68（マクロファージの表面マーカー）の発現が好酸球群で有意に亢進していた．IL-8の発現は好酸球群と好中球群ともに非好酸球性・非好中球性群よりも亢進していた．Cu/Zn SODは好酸球群で他の2群に比して発現の低下を認めた．さらに，好酸球性副鼻腔炎はアスピリン不耐性の有無，アスピリン耐性は好酸球性ムチンの有無で臨床像や免疫学的背景に相違があることが判明し，図2のような細分類を提唱する．

好酸球性副鼻腔炎の病態はまだ十分に解明されていない．しかしながら，喘息を合併する場合が多く，また喘息の発症前の症例も多く含んでいるため，喘息との共通した病態が示唆されている．好酸球性副鼻腔炎の病因としては，①黄色ブドウ球菌などの内毒素由来のスーパー抗原，②真菌のⅠ型アレルギー，③真菌の非IgE依存性のアレルギー反応，④アスピリン不応性が提唱されている[11]．

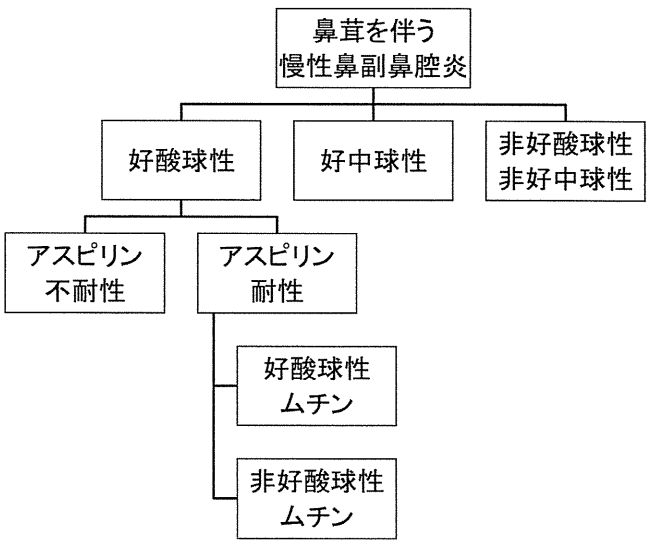

図2　鼻茸を伴う慢性鼻副鼻腔炎の臨床的細分類

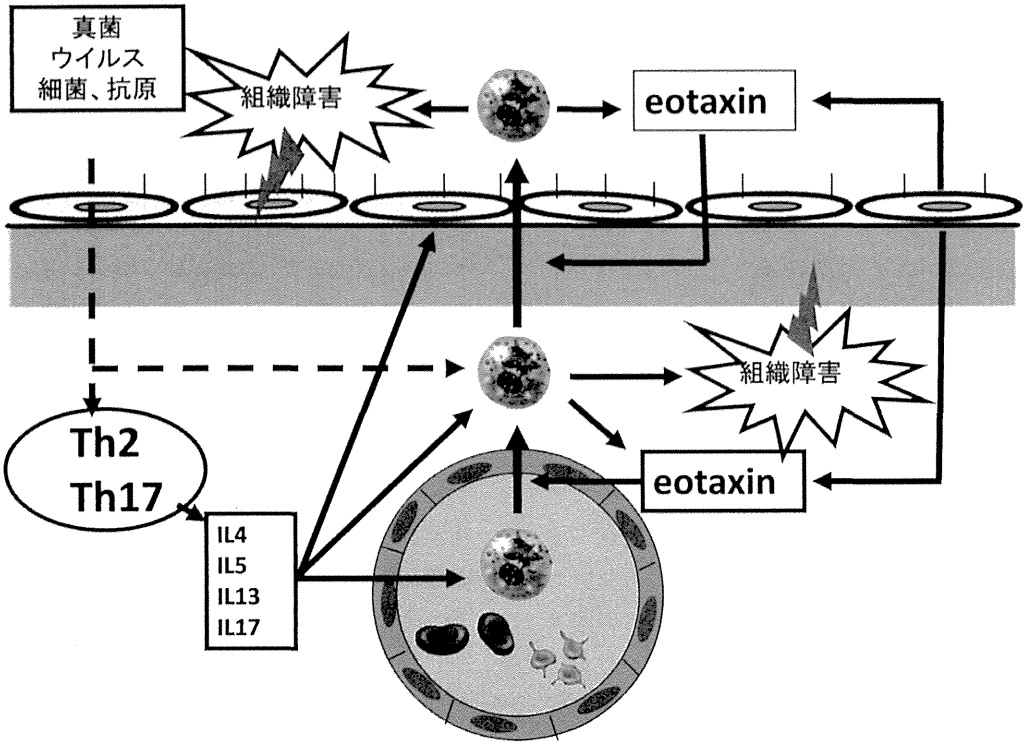

図3　好酸球の選択的動員のメカニズム

一方，近年ステロイド抵抗性で好中球浸潤を伴う難治性喘息に新しいヘルパーT細胞パラダイムであるTh17の関与が指摘されている．喘息を合併した鼻ポリープを伴う慢性副鼻腔炎にはIL-17A陽性細胞が著明に存在した．IL-17Aの陽性細胞は主にCD4陽性リンパ球と好酸球であった．Th17A陽性・CD4陽性細胞数は粘膜に浸潤する好酸球数とよく相関していた．これらの事実より好酸球性副鼻腔炎の好酸球浸潤には従来から提唱されていたTh2に加えて，Th17の関与が推察される[22]．外的因子によって，Th2とTh17系が活性化され，IL-4，IL-5，IL-17などのサイトカインが放出され，標的細胞を刺激して，RANTESやeotaxinの産生[23]を促進し，好酸球の選択的な動員や粘膜のリモデリング[24]が生じると推察される（図3）．

保存的治療のエビデンス

好酸球性副鼻腔炎の病態を基盤として，作用機序に沿った治療が試みられている．有効とされている治療法，無効とされている治療法，術後の治療法に関して，症状への効果，客観的検査の評価，鼻ポリープへの効果，エビデンスレベルを表1～4に示す[2]．

ステロイド噴霧薬の鼻腔局所治療やステロイドの全身

表1　好酸球性副鼻腔炎の保存的治療法（有効）その1（文献2から改変）

治療法	症状への効果	客観的検査の評価	鼻ポリープへの効果	エビデンスレベル
鼻用ステロイド噴霧薬（4～16週）	鼻閉，嗅覚，鼻漏の改善	鼻腔通気度の改善	縮小	Ib
ステロイドの全身投与（2週）	改善	記載なし	縮小	Ib，III
マクロライドの長期投与（3ヵ月）	急性増悪の低下，症状改善	サッカリンテスト，NOの改善	・ポリープ症例には適応外（Ib） ・縮小（好中球性，III） ・ポリープ・非ポリープ症例で有意差なし（Ib）	Ib，III

表 2　好酸球性副鼻腔炎の保存的治療法（有効）その 2（文献 2 から改変）

治療法	症状への効果	客観的検査の評価	鼻ポリープへの効果	エビデンスレベル
混合死菌	効果あり	効果なし （急性増悪の低下）	治験なし	Ib
フロセミド吸入	効果あり	評価なし	効果あり （ステロイド内服と同等）	Ib
抗ロイコトルエン拮抗薬	改善	不変	改善	III
カプサイシン	評価なし	CT で効果あり	効果あり	III
抗菌剤（急性増悪時）	効果あり	評価なし	評価なし	IV

表 3　好酸球性副鼻腔炎の保存的治療法（無効）（文献 2 から改変）

治療法	症状への効果	客観的検査の評価	鼻ポリープへの効果	エビデンスレベル
血管収縮薬	効果なし	効果なし	効果なし	なし
粘液融解剤，免疫調節剤， 漢方，PPI，鼻洗浄	治験なし	治験なし	治験なし	なし
抗ヒスタミン薬	アレルギー症状の改善	なし	なし	Ib，III
抗真菌薬（洗浄，8 週～ 6ヵ月）	効果なし	CT，内視鏡所見 などで効果なし	効果なし	Ib
抗 IL-5 抗体	効果なし	評価なし	効果なし	Ib

表 4　好酸球性副鼻腔炎の術後治療法（文献 2 から改変）

治療法	症状への効果	客観的検査の評価	鼻ポリープ再発への効果	エビデンスレベル
鼻用ステロイド噴霧薬 （26 週～ 5 年）	鼻閉，くしゃみ， 鼻漏の改善	鼻腔通気度の改善	効果あり	Ib
ステロイドの全身投与（2 週）	改善	記載なし	縮小	Ib，III
アスピリン減感作 （全身，局所）	改善	記載なし	効果あり	Ib
カプサイシン	評価なし	評価なし	効果あり	Ib
抗ヒスタミン薬	アレルギー症状の改善	記載なし	効果なし	Ib
フロセミド吸入	改善	評価なし	効果あり	IIa
抗ロイコトルエン拮抗剤	改善	評価なし	効果あり （ステロイド点鼻と同等）	IIa

投与は高いエビデンスで有効とされている．マクロライド長期投与はポリープへは適応外であり，好中球性病変に奏功することが一般に是認されている．

血管収縮剤の局所投与や抗ヒスタミン剤は効果なく，粘液融解剤，免疫調整剤，漢方，プロトンポンプ阻害剤，鼻洗浄では効果判定の検討はなされていない．抗真菌剤による鼻洗浄の有効性は否定されており，同時に慢性鼻副鼻腔炎における真菌病因説も否定されている．抗 IL-5 抗体療法は症例によっては鼻ポリープに有効とされているが，統計学的な有意差はない．

術後治療法として，保存的治療法で有効であるステロイド噴霧薬の鼻腔局所治療やステロイドの全身投与，フロセミド吸入，抗ロイコトルエン拮抗剤，カプサイシン局所投与に加えて，アスピリン過敏性の鼻ポリープに対するアスピリンの減感作療法の有効性も報告されている．

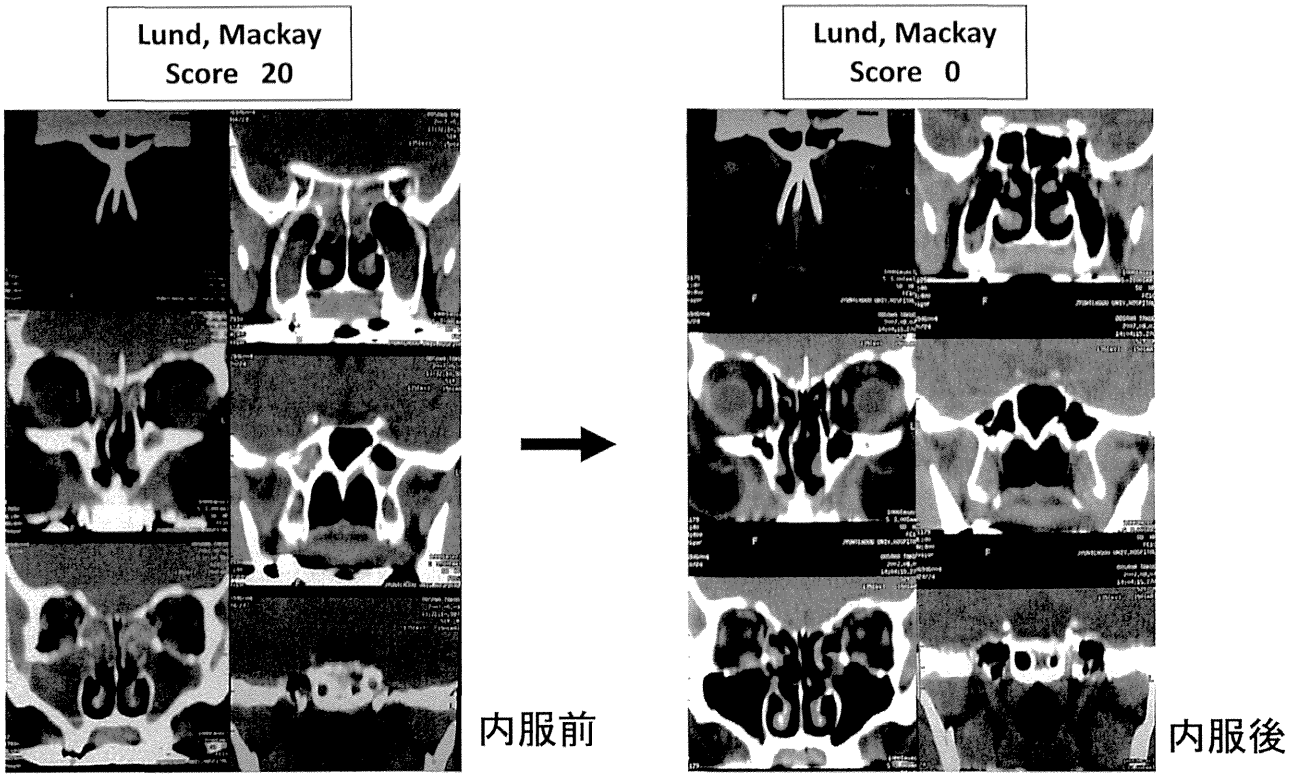

図 4　ステロイド内服前後の鼻副鼻腔 CT 像

当科における治療戦略

一般に保存的治療法が第一選択であり，保存的治療に抵抗を示す重症例が手術適応となる[25]．当科での好酸球性副鼻腔炎の治療指針は，血中好酸球の増加，鼻汁スメアの好酸球の存在，篩骨洞・嗅裂部を中心とした高度粘膜病変，早期からの嗅覚障害などの臨床ならびに検査所見を呈する場合や鼻ポリープの生検で好酸球の集簇を認めた場合は好酸球性副鼻腔炎と診断する．軽・中等症は抗ロイコトルエン拮抗剤，経口または局所ステロイド剤を行い，治療困難または再発を繰り返す症例ならびに重症例に対して，手術治療（内視鏡下副鼻腔手術，ESS）を選択する．可能であれば術前後にステロイド剤を内服投与する．ESS はマイクロデブリッターを用いてポリープを除去し，上鼻甲介の下半分を切除し，嗅覚路を確保し，すべての副鼻腔を可及的に大きく開洞する．術後は抗アレルギー剤と鼻内吸入ステロイド剤の長期投与と鼻洗浄で管理する．

術後の管理にはニオイスティック（香水）を用いて，self smell test（SST，自己嗅覚検査）によって嗅力の有無を自己判定・評価する．再発の徴候の判断は，嗅覚の低下と感染である．SST で嗅力の消退やニカワ様の好酸球性ムチンを示唆する鼻漏の出現時ではプレドニゾロン

表 5　ステロイドの全身投与に対する上下気道の感受性

		鼻副鼻腔	
		応答性（80%）	不応答性（20%）
気管支	応答性	13	6
	不応答性	2	2
	喘息なし	2	0

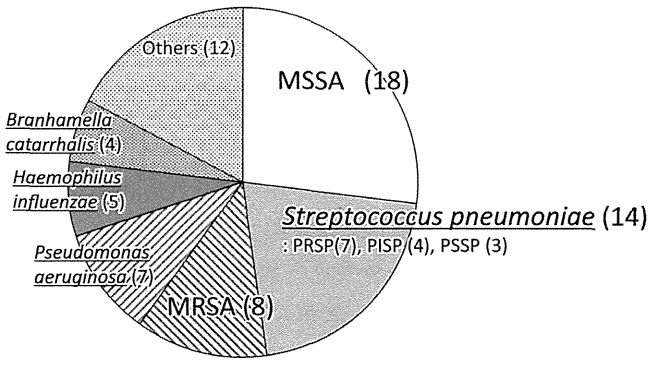

図 5　術後の急性増悪時の鼻副鼻腔から検出された細菌の種類と割合（文献 31 から改変）

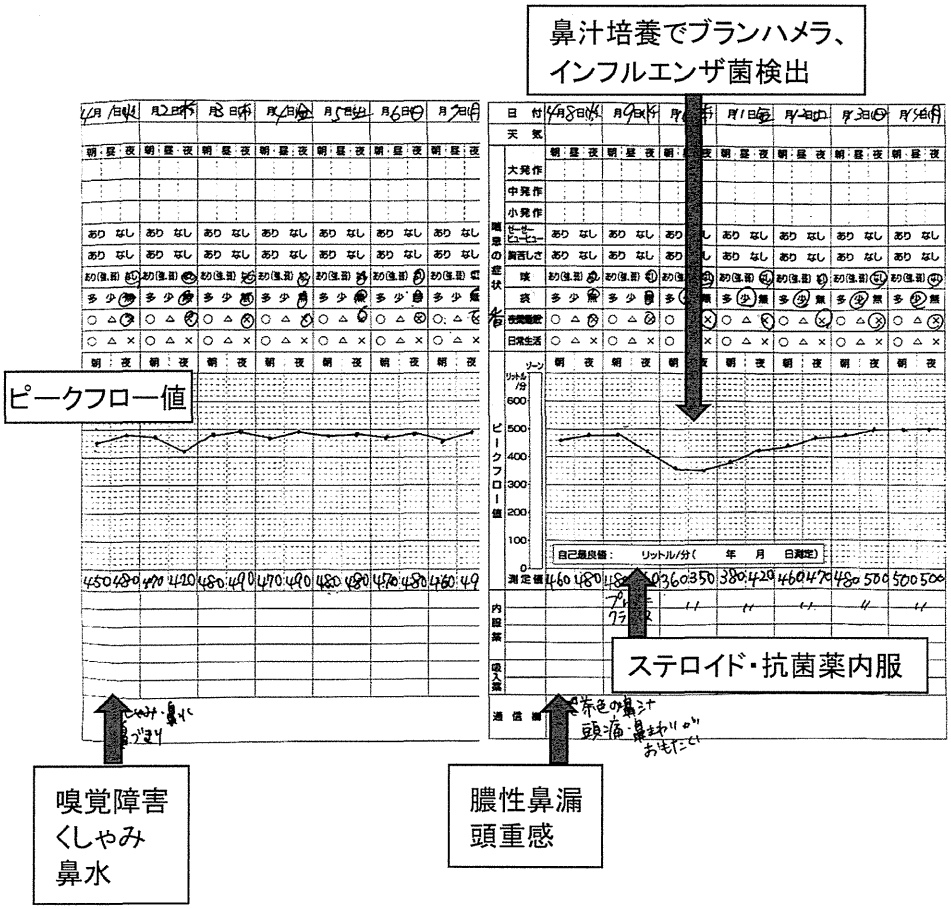

図6　上気道炎を契機として，副鼻腔炎ならびに喘息の増悪をきたした症例のピークフローの継時的変化

(0.5 mg/kg) を，膿性～膿粘性鼻漏が持続する場合では抗菌薬（主にレスピラトリーキノロン）を頓服させる．また喘息合併症例や下気道の症状の合併症例ではピークフローメーターによる下気道の管理も指導している．上・下気道の所見を可能な限り日記として記載してもらい，指導管理の資料としている[26]．図4は術前にステロイド剤を全身投与した症例の投与前後のCT像である．無治療症例群（対照）とステロイド全身投与群のCTスコアの変化では有意な差を示し，ステロイド全身投与の有効性を認めた．またポリープ組織の好酸球数も低下した[27]．

40例で術後のフォローアップの経過中にポリープの再発を認めた25例にステロイドの頓服を行った．その内，8例（20%）ではステロイドの感受性が悪かった．一方，喘息のステロイド感受性は応答が19例，不応答が4例であった（表5）．

再発の重要な要因として細菌感染がある．その理由として，①副鼻腔の細菌叢が喘息の感染増悪の温床となること[28]，②急性副鼻腔炎の3大起因菌（肺炎球菌，インフルエンザ菌，モラクセラ・カタラーリス）が喘息増悪の要因となること[29]，③喘息の70～75%で上気道にモラクセラ・カタラーリスを検出することである[30]．術後で膿性鼻漏などの再発徴候を示した37例から83株の細菌が検出された．正常細菌または菌株なしは5例であった．急性増悪の起因菌として肺炎球菌，インフルエンザ菌，カタラリス菌は23株認めた（図5）．上気道炎を契機として，副鼻腔炎の急性増悪を生じ，ピークフローメーター値の低下を示した（図6）．ピークフローメーター値を計測した24例中6例に低下を認めた．全6例で急性副鼻腔炎の3大起因菌または緑膿菌を検出した[31]．以上より，鼻副鼻腔の細菌感染は副鼻腔炎のみならず，喘息の再燃に関与することが確認され，喘息合併症例では副鼻腔炎の再燃の防止は喘息の良好な経過にも貢献する．

結　語

エビデンスに基づく慢性鼻副鼻腔炎の保存的治療法，手術治療，術後管理の戦略を選択することが求められる．

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〒113-8421 東京都文京区本郷2-1-1
順天堂大学医学部耳鼻咽喉科学講座

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Reduction in Superoxide Dismutase Expression in the Epithelial Mucosa of Eosinophilic Chronic Rhinosinusitis with Nasal Polyps

N. Ono T. Kusunoki M. Miwa M. Hirotsu A. Shiozawa K. Ikeda

Department of Otorhinolaryngology, Juntendo University Graduate School of Medicine, Tokyo, Japan

Key Words
Chronic rhinosinusitis · Eosinophils · Nasal polyp · Superoxide dismutase

Abstract
Background: Eosinophils generate large amounts of oxidant species. The eosinophil-dominant type of chronic rhinosinusitis (CRS) with nasal polyps is related to more extensive disease and a decreased likelihood of surgical success. Superoxide dismutase (SOD) is the first-line and only antioxidant enzyme that converts superoxide to hydrogen peroxide. ***Methods:*** The patients with CRS with nasal polyps were divided into eosinophilic and noneosinophilic groups. The expression of three isoforms of SOD, intracellular copper-zinc SOD (CuZnSOD), mitochondrial manganese SOD (MnSOD) and extracellular SOD (ECSOD), were examined by enzyme activity assay, immunohistochemistry and quantitative real-time RT-PCR sampled by laser capture microdissection. ***Results:*** SOD activity in the eosinophilic and noneosinophilic groups was significantly reduced compared to that of the control groups. Immunostaining of both CuZnSOD and MnSOD in the eosinophilic group was significantly decreased compared with that in the noneosinophilic and control groups. CuZnSOD mRNA of the eosinophilic group was significantly decreased compared with that of the control group, whereas MnSOD mRNA in the eosinophilic group was significantly decreased compared with that in the noneosinophilic and control groups. Neither immunoreactivity nor mRNA of ECSOD was different among the three groups. The degree of epithelial damage and disease severity were inversely correlated with CuZnSOD and MnSOD immunoreactivity. ***Conclusions:*** The reduction in SOD activity and the downregulation of the SOD message are suggested to be related to eosinophil recruitment and epithelial damage of CRS with nasal polyps.
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Introduction

Chronic rhinosinusitis (CRS) is defined as persistent inflammation of the nasal and paranasal cavity mucosa lasting ≥3 months [1]. Based on an epidemiological study, CRS affects 12.5% of the population in the United States [2]. The prevalence and medical costs of CRS are increasing and have become important social issues.

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. CRS with nasal polyps associated with mucosal infiltration of eosinophils may be regarded as eo-

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Correspondence to: Dr. Katsuhisa Ikeda
Department of Otorhinolaryngology
Juntendo University Graduate School of Medicine
2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421 (Japan)
E-Mail ike@juntendo.ac.jp

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sinophilic CRS due to the distinctive feature of tissue eosinophilia [3], which is more refractory to surgical treatment and is frequently associated with bronchial asthma. The histopathological features of asthma, including tissue eosinophils, epithelial damage and basement membrane thickening of the lower airways, are also observed in sinonasal specimens with CRS [4]. Epithelial damage in asthma is thought to be a consequence of ongoing inflammation, bronchoconstriction and host susceptibility factors [5]. We found a significant correlation between basement membrane thickness and eosinophilic infiltration into both the epithelial and subepithelial layers of the nasal polyps of CRS [6].

Eosinophils have some cytotoxic mediators such as eosinophilic peroxidase, which can be produced during oxidative stress, resulting in severe damage to the epithelia [7]. The role of oxidative stress in the pathogenesis of nasal polyps has been recognized recently. High levels of malondialdehyde, one of the metabolites of free radical-mediated lipid peroxidation, were observed in nasal polyps [8, 9]. Moreover, free radical levels in nasal polyps were associated with nasal polyp severity [10, 11]. The overproduction of free radicals and decrements in the antioxidant system can both cause tissue injury. We previously demonstrated that heme oxygenase-1, a cytoprotective enzyme against oxidant, is reduced in epithelial cells in CRS with eosinophilic infiltration [12].

Superoxide dismutase (SOD) is the most important antioxidant enzyme system against free radicals and the decomposition of superoxide radicals into H_2O_2 in the human lung. SODs are composed of three isoforms of intracellular copper-zinc SOD (CuZnSOD), mitochondrial manganese SOD (MnSOD) and extracellular SOD (ECSOD). Previous studies regarding the measurement of SOD activities in nasal polyps are still controversial. Karlidag et al. [13] and Cannady et al. [14] reported that the enzyme activity of SOD was lower in the nasal polyps than in the normal inferior turbinate. In contrast, SOD activity has been reported to be unchanged in the nasal polyps compared with the normal middle turbinate. Cheng et al. [15] reported that the expression profiles of SOD isoforms could be determined by reverse transcription-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay and Western blotting, and found that the expression of both CuZnSOD and ECSOD was higher in polyp tissue. However, previous reports did not focus on eosinophilic infiltration into nasal polyps. Herein, we examined the enzymatic activity, localization of the protein and expression of the message of SOD from the viewpoint of eosinophilic inflammation. Furthermore, we analyzed the relationship between SOD expression and epithelial damage. This study will provide a helpful key to determining whether SOD would be a protective factor for eosinophilic CRS and a new tool for ameliorating eosinophilic CRS.

Patients and Methods

Patients

Cases of CRS with nasal polyps were diagnosed based on the criteria of the European position paper [16]. The disease extent on computed tomographic (CT) scans was categorized according to Lund and Mackay [17]. None of the patients were treated with systemic corticosteroids or other immune-modulating drugs for at least 1 month before surgery. All patients gave their written informed consent, and the study was approved by the Ethics Committee of the Juntendo University Faculty of Medicine.

Sampling of Tissue Specimens

Tissue specimens were obtained from nasal polyps located in the middle meatus. Control samples were obtained from normal mucosal membranes of the sphenoid sinus removed during surgery for pituitary adenoma. A part of each sample was fixed in 10% formalin embedded in paraffin wax, processed routinely and stained with hematoxylin-eosin. The patients were classified into 2 groups according to our recent report [18]. The eosinophilic and noneosinophilc groups were defined as eosinophil counts of the nasal polyp of more and less than 100/microscopic field (magnification: ×400) using 3 fields, respectively. Eosinophils were quantified in the foci of the densest cellular infiltrate. The length of epithelial sloughing was expressed as a percentage of the total epithelial length. The residual sample tissue of the nasal polyps was used for the enzyme activity assay, immunohistochemistry and quantitative real-time RT-PCR. However, due to the amount of the samples or the conditions of preparation, these three series of experiments could not be performed using samples from all the patients.

SOD Activity

The tissue samples were homogenized using a bead-based homogenizer. After centrifugation at 78,000 *g* for 60 min at 4°C, the upper clear layer was taken. The total protein concentration was measured by a Pierce 660-nm protein assay reagent (Thermo Scientific Pierce, Japan). SOD activity was assayed using an SOD assay kit (WST; Dojindo Laboratories, Japan) according to the manufacturer's instructions.

Immunohistochemistry

Nasal polyps were fixed in 10% formalin, embedded in paraffin wax, processed routinely and then prepared as routine semithin sections (3.5 μm). The primary antibodies were CuZnSOD 1:200, MnSOD 1:500 and ECSOD 1:2,000. Rabbit anti-human CuZnSOD and MnSOD was obtained from Stressgen Bioreagents (USA). Rabbit anti-human ECSOD was obtained from Abnova (USA). The sections were stained by a Ventana iVIEW DAB detection kit using a Ventana (USA) automated stainer (NexES IHC). The sections treated with control rabbit and mouse IgG1 served as negative controls.

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Table 1. Clinical and epidemiologic profiles of the study groups

	Eosinophilic (n = 31)	Noneosinophilic (n = 28)	Control (n = 20)	p value
Age, years	49.5±12.3	49.8±18.8	60.9±17.0	
Gender, male/female	21/10	18/10	10/10	
Symptomatic score	11.8±2.43	8.91±2.21		<0.01
CT score	19.7±5.08	12.7±4.61		<0.001
Peripheral blood eosinophils, n/μl	640.1±303.9	143.0±118.2		<0.001
Tissue eosinophils, n	325.7±198.7	23.2±24.4		<0.001

Laser Capture Microdissection

Frozen sections, microdissected (8 μm) on a laser capture microdissection system (Leica DMLA, Leica Microsystems, Germany), were immediately fixed in 99.5% ethanol. At least 500 epithelial cells were microdissected from cryosections.

Quantitative Real-Time RT-PCR

Total epithelial RNA was extracted from the microdissected samples using an RNeasy micro kit (Qiagen, Germany). cDNA was synthesized from total epithelial RNA with the PrimerScript II first-strand cDNA synthesis kit (TAKARA Bio, Japan). Real-time RT-PCR was performed for CuZnSOD, MnSOD and ECSOD using a 7500 fast real-time PCR system (Applied Biosystems, USA) and TaqMan gene expression assays (assay identification Nos. Hs00533490_m1, Hs00167309_m1 and Hs00162090_m1) according to the manufacturer's specifications. Data analysis was done on Applied Biosystems 2.0.1 software. All results were normalized to β-actin to compensate for differences in the amount of cDNA (assay identification No. Hs99999903_m1).

Statistical Analyses

Data were expressed as means ± SD. Student's t test was used for age, gender, symptomatic score, CT score, and eosinophils in the serum and tissue. One-way ANOVA was used for SOD activity, immunohistochemistry and quantitative real-time RT-PCR. Pearson's correlation coefficient was applied to assess the relationships among the rate of SOD-positive epithelial cells, CT score and degree of epithelial damage. Statistical analysis was performed using State Mate IV for Windows. * $p < 0.05$ was considered as statistically significant.

Results

Clinical Background

Clinical and epidemiologic data are shown in table 1. There were no significant differences in age or gender among the eosinophilic, noneosinophilic and control groups. Four clinical parameters such as symptomatic score, CT score, blood eosinophils and tissue eosinophils were significantly higher in the eosinophilic group than in the noneosinophilic group.

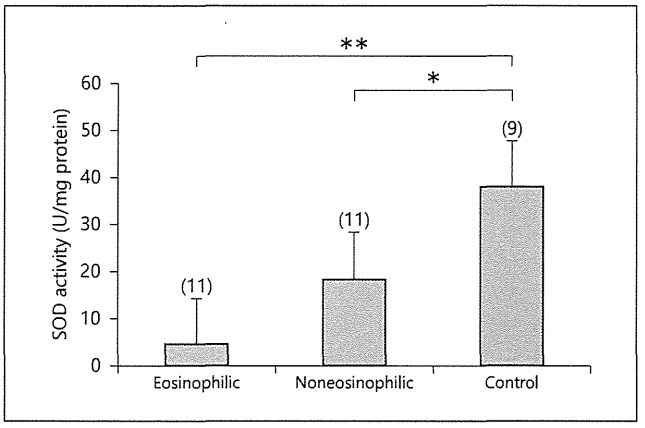

Fig. 1. Decreased activity of SOD in eosinophilic and noneosinophilic CRS with nasal polyps compared with the control group. Numbers of samples are given in parentheses; * $p < 0.05$; ** $p < 0.01$.

SOD Activity

SOD activity was examined in the eosinophilic and noneosinophilic CRS with nasal polyps as well as control sinus mucosa (fig. 1). SOD activity of the eosinophilic and noneosinophilic groups was significantly lower than that of the control group. The eosinophilic group tended to show reduced SOD activity compared with the noneosinophilic group, but no significant difference was found.

Immunohistochemical Study

The labeling localization and distribution of the three SOD isoforms were determined using immunohistochemistry (fig. 2). CuZnSOD immunoreactivity was mainly observed in the epithelium, fibroblasts and vascular endothelium in normal sinus mucosa. In the epithelium, CuZnSOD was expressed most prominently in ciliated epithelial cells. MnSOD was moderately to highly expressed in epithelial cells and vascular endothelium.

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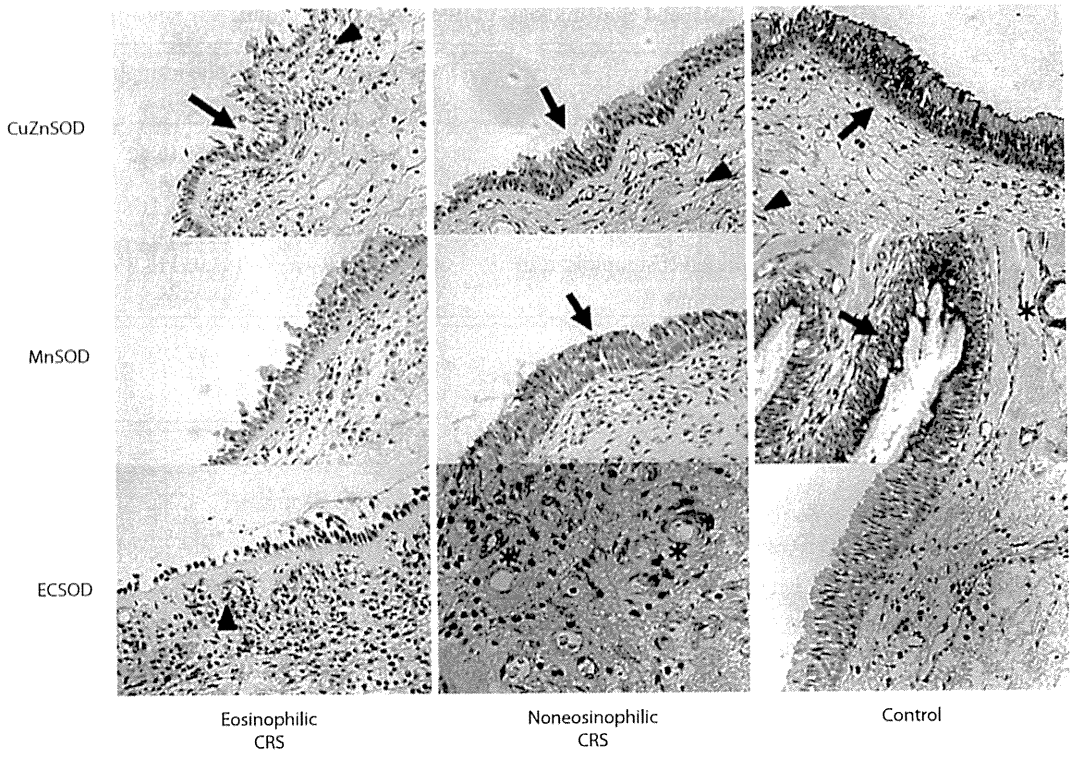

Fig. 2. Histochemical localization and distribution of SOD in nasal polyps. Arrows indicate epithelial cells; arrow heads fibroblast, and asterisks indicate vascular endothelium.

On the other hand, weak expression of ECSOD was observed in fibroblasts and vascular endothelium as well as epithelial cells. Immunoreactivity for SOD isoforms was semiquantitatively assessed in epithelial cells (fig. 2, 3). A significant reduction in immunopositivity for CuZnSOD and MnSOD in eosinophilic CRS was recognized in comparison with the noneosinophilic and control groups (fig. 3a, b). In contrast, there was no difference in ECSOD among the three groups (fig. 3c).

Real-Time Quantitative PCR

In order to obtain selective sampling of the cells enriched with target molecules, we applied laser capture microdissection to histological sections of nasal polyps. Information on the mRNA expression of SOD in epithelial cells by real-time quantitative PCR is shown in figure 4. The CuZnSOD mRNA levels in eosinophilic CRS were significantly decreased compared with those of the controls (fig. 4a). Although not statistically significant, the CuZnSOD mRNA levels in the eosinophilic group tended to be lower than those of the noneosinophilic group. A significant downregulation in the MnSOD message was seen in the eosinophilic group compared with those of the other two groups (fig. 4b). ECSOD mRNA in epithelial cells showed no difference among the three groups (fig. 4c).

Relevance of SOD Expression to Disease Severity and Epithelial Damage

CT scores were significantly and inversely correlated with the reduction in SOD activity in CRS with nasal polyps. The correlation between immunopositivity for CuZnSOD and MnSOD with the degree of epithelial damage was evaluated using all samples from CRS with nasal polyps. The epithelial damage had a significantly inverse correlation with the rate of both CuZnSOD- and MnSOD-positive epithelial cells (fig. 5).

Discussion

The present study first and clearly demonstrated that eosinophilic infiltration and resultant tissue damage in CRS with nasal polyps is characterized by a disruption

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