

Original Article

Comparison of local cytokine gene expression and the distribution of eosinophils and CD4-positive cell subsets in the paranasal sinus mucosa between atopic and non-atopic subjects

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ABSTRACT

Background: The role of Th2 type cytokines in the persistence of chronic rhinosinusitis, especially that induced by non-infectious inflammatory causes, has been noted. However, the original cause of sinus eosinophilia remains unclear and whether the presence of allergic rhinitis (AR) may be a risk factor has been an issue of debate. In the present study, we examined cytokine expression and the distribution of CD4-positive cell subsets in the paranasal sinus mucosa of patients with chronic sinusitis.

Methods: A total of 133 sinusitis patients was examined. Patients were subdivided into four groups based on the presence of AR and the degree of local eosinophil infiltration. The expression of granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-5, IL-8, IL-16, eotaxin and interferon (IFN)- γ mRNA was detected by reverse transcription-polymerase chain reaction. Immunohistochemical localization of CD4-, CXCR3- and CCR4-positive cells

in the same specimens was quantitatively analyzed using a laser scanning confocal microscope.

Results: The group of non-AR patients with low eosinophilia (the AR(-)Eo(-) group) only showed an increase in IFN- γ mRNA expression. In contrast, the other three groups showed similar cytokine profiles, with high expression levels for GM-CSF, IL-5 and eotaxin mRNA. The total number of CD4⁺ cells was also increased in these three groups. The density of CCR4-positive CD4⁺ cells was significantly higher in groups with high eosinophilia, irrespective of the presence of AR. As a result, the CXCR3⁺/CCR4⁺ cell ratio in the AR(-)Eo(-) group was significantly increased compared with the other three groups.

Conclusions: These results indicate that high expression of Th2-type cytokines concomitant with the infiltration of a predominant number of CD4⁺ cells and their Th2 subsets play a role in the pathogenesis of eosinophil inflammation in sinus mucosa. In addition, the finding that some of the non-atopic patients also shared Th-2 type immune responses provides support for the concept of chronic sinusitis as a Th2-mediated disease process.

Key words: allergic rhinitis, CCR4, CD4, chronic sinusitis, CXCR3, cytokines, eosinophils.

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INTRODUCTION

The overall pathological view of paranasal sinus infection has profoundly changed and evolved in recent

decades. In addition, a great deal of knowledge about the immunopathology of chronic sinusitis has accumulated. The importance of sinonasal anatomic variants in creating a predisposition to sinus disease through blockage of the ostiomeatal complex has become a subject of controversy. Contrary to common belief, recent studies have failed to present a significant association between the recurrence of chronic sinusitis and most of these anatomic variants.¹ However, a close correlation has been noted between systemic or local eosinophil recruitment and the extent of sinus disease, as evaluated by computed tomography.² Eosinophils are considered to play a major role in the pathogenesis of chronic sinusitis through the release of noxious secretory granules.³ Several reports have debated the role of nasal allergen sensitization, as well as the increased incidence of an atopic population.⁴⁻⁹ However, the original cause of eosinophil recruitment in chronic sinusitis remains controversial and several authors have claimed that atopic status does not affect eosinophil infiltration in the sinus mucosa.¹⁰⁻¹²

In the present study, we performed a molecular and immunohistologic analysis of paranasal sinus mucosa in patients with chronic sinusitis. Patients were subdivided into four groups based on the presence of allergic rhinitis (AR) and the degree of local eosinophil infiltration. We examined local expression of a panel of cytokine mRNA and quantitatively analyzed the proportion of CD4-, CXCR3- and CCR4-positive cell subsets using the same specimens. The presence of CD4⁺ lymphocytes within the sinus mucosa has attracted considerable attention because these cells are capable of promoting antigen-specific inflammatory responses through the secretion of specific cytokines and chemokines, leading to the promotion of inappropriate inflammatory cell infiltration.¹³ The respiratory epithelium has also been shown to be an essential target of inflammatory attack by activated T cells and eosinophils.¹⁴ Recent studies have shown that the cells capable of producing Th2 cytokines are restricted to the CCR4-expressing population within memory CD4⁺ T cells, whereas interferon (IFN)- γ -producing cells reside exclusively in CXCR3-expressing CD4⁺ T cells.¹⁵⁻¹⁹

Allergic status is now considered as a disorder of the whole respiratory tract, not limited to a specific target organ. In this sense, the pathophysiological features of chronic sinusitis in allergic subjects can be considered to be dependent on the effects of Th2 cytokines.²⁰⁻²² Very limited information is available thus far on the expression

and function of these specific chemokine receptors during the inflammatory response in paranasal sinuses. We consider the present study to be a valuable one in assessing the relative weight and role of allergies involved in eosinophil infiltration into the sinus mucosa, compared with other possible mechanisms, among the Japanese population.

METHODS

Subjects

The primary method of the present investigation was immunohistologic and molecular analysis of sinus mucosa specimens from patients with chronic sinusitis stratified by allergic status and local eosinophil infiltration. We defined a patient whose sinus specimen showed more than 300 eosinophils/mm² as exhibiting high eosinophil infiltration. A total of 133 sinusitis patients were divided into four groups, as follows: (i) non-AR patients with low eosinophil infiltration (AR(-)Eo(-); $n = 62$); (ii) non-AR patients with high eosinophil infiltration (AR(-)Eo(+); $n = 15$); (iii) AR patients with low eosinophil infiltration (AR(+)Eo(-); $n = 41$); and (iv) AR patients with high eosinophil infiltration (AR(+)Eo(+); $n = 15$). Chronic sinusitis was diagnosed by nasal endoscopies and computed tomography. All patients had shown the presence of persistent sinus-related symptoms for more than 3 months. None of the patients had received topical or systemic steroids for at least 4 weeks before surgery. Allergic rhinitis was diagnosed by clinical and laboratory findings. All patients had a score of 2 or more on a CAP-RAST test to at least one of the common inhaled allergens. Most showed positive scores against house dust extracts or *Dermatophagoides farinae*.

Sinus mucosa specimens were obtained from the most inflamed region of the maxillary sinus or ethmoid cells at the time of sinus surgery. In each case, specimens were divided and either fixed in fresh 4% paraformaldehyde for immunohistochemistry or, alternatively, immersed in RNA later™ solution (Ambion, Austin, TX, USA) for reverse transcription-polymerase chain reaction (RT-PCR). The study protocols were reviewed and approved by the local ethics committee at the University Hospital, Hiroshima. All subjects provided written informed consent to participate in the study.

Analysis of cytokine mRNA expression

Total cellular RNA was extracted by ice-cold Trizol reagent (Gibco BRL, Rockville, MD, USA) according to the instructions supplied by the manufacturer. Contaminating DNA was removed using deoxyribonuclease I (amplification grade; Gibco BRL). Five micrograms of the RNA was then reverse-transcribed to cDNA using the Superscript First-Strand Synthesis System (Gibco BRL). The reaction was performed in a mixture of RT buffer, 4 μ L of 100 mmol/L dithiothreitol, 2 μ L of 10 mmol/L dNTP mix, 80 units RNase inhibitor, 1 μ g oligo(dT) primers and 100 units Superscript II RT to a total volume of 40 μ L. The RT mixture was incubated for 5 min at 65°C for initial annealing, followed by 50 min at 42°C for RT and 15 min at 70°C for termination.

An RT-PCR procedure was performed to determine relative quantities of mRNA for the human granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-5, IL-8, IL-16, eotaxin and IFN- γ genes using an adaptation of methods described elsewhere.²³ Briefly, an aliquot of the RT product was subjected to PCR amplification in the presence of a mixture containing PCR buffer, MgCl₂, dNTP mix, rTaq polymerase and paired primers to a total volume of 30 μ L. The PCR conditions and the oligonucleotide primers for GM-CSF, IL-5, IL-8, IL-16 and eotaxin have been described previously.²³ The primer pairs specific for human IFN- γ were customarily prepared based on the sequences described elsewhere.²⁴ To verify that equal amounts of cDNA were added to each PCR amplification, primers for the housekeeping gene β -actin were also used in each experiment. The number of PCR cycles was 30 for β -actin and 35 for the others, using an MJ Research PTC-100 thermal cycler (Watertown, MA, USA). The PCR products (10 μ L) were analyzed in 1.5% agarose gels containing ethidium bromide. They were verified using molecular weight markers (Bio Laboratories, Beverly, MA, USA) and the luminescence intensity was measured with a charge-coupled device image analyzer (AE-6915; Atto, Tokyo, Japan). The level of cytokine expression was described as the ratio relative to the intensity of β -actin in the same cDNA.

Fluorescence immunohistochemistry

Antihuman CD4 mouse monoclonal antibody (MT310) was from Dako (Glostrup, Denmark); antihuman CXCR-3 goat polyclonal antibody (C-20) and antihuman CCR-4 rabbit polyclonal antibody (H-48) were

from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All Alexa Fluor-conjugated secondary antibodies for immunofluorescence were from Molecular Probes (Eugene, OR, USA).

Immunofluorescent staining was performed on 8 μ m cryostat sections from each specimen displaced in adjacent series on slides. At least three series were used for each staining. Sections were fixed in cold 2% paraformaldehyde solution, followed by washing in phosphate-buffered saline (PBS), and were treated with a solution of 20 mmol/L HEPES, 300 mmol/L sucrose and 0.5% Triton X-100 for 30 min, as described previously.²³ After blocking with 1% bovine serum albumin, sections were incubated overnight at 4°C in the presence of the primary antibodies. The slides were rinsed with PBS and incubated with the Alexa Fluor-conjugated secondary antibodies (Alexa Fluor 488 goat antirabbit IgG, Alexa Fluor 488 donkey antigoat IgG or Alexa Fluor 568 goat anti-mouse IgG) for 1 h at room temperature. After washes in PBS, slides were mounted with 90% glycerol/PBS. Double labeling with primary antibodies from different host species (CD4-CXCR3 and CD4-CCR4) was performed in the present study. Negative controls were performed omitting the primary antibody or using an isotype control antibody from the same species. Consecutive sections were stained with hematoxylin and eosin (HE) in order to view the mucosal pathology.

For quantitative analysis, simultaneous two-color fluorescence images were recorded using a laser scanning confocal microscope (LSCM; Leica TCS-NT; Leica Microsystems, Heidelberg, Germany) equipped with an argon-krypton laser at wavelengths of 488 and 568 nm. Five images of each section were obtained at $\times 400$ magnification (0.0625 mm²/frame). Cell counts were performed in each field from randomly selected sections with the use of an image-analysis program (Scion Image; Scion, Frederick, MA, USA) blind to the clinical diagnosis.

Data analysis

Categorical variables were analyzed with the Chi-squared test. Group data were expressed as the mean $\pm$ SEM or as a median and interquartile range when appropriate. For multiple comparisons, screening of data for differences was first performed using the Kruskal-Wallis test. If the analysis gave a significant result, further comparison of individual data was undertaken by the Mann-Whitney U-test for between-group analysis. Statistical analysis was performed with

STATISTICA for Windows (StatSoft, Tulsa, OK, USA). Probability values of $P < 0.05$ were accepted as significant.

RESULTS

Patient characteristics

The present study aims to portray the histopathological features of chronic sinusitis with emphasis on the difference in eosinophil infiltration. Table 1 shows the background and histological characteristics of the study population. We found that the overall distribution of eosinophil density in all patients has shown a combination of two different standardized shapes. Therefore, we set the density of 300 eosinophils/mm² as a value to demarcate the two groups.²⁵ In allergic patients, 15 of 56 patients (26.7%) had shown remarkable tissue eosinophilia in their sinus mucosa, which was a higher percentage than in non-allergic patients (19.4%), although the proportion was not significantly different. Patients in the AR(+)Eo(-) group were significantly younger than in the other three groups. In this group, there were also more men, with a respective male : female sex ratio of 4.12, although the difference was not significant. Patients in the AR(-)Eo(+) and AR(+)Eo(+) groups showed significant increases in peripheral blood eosinophils. We also screened the density of neutrophils and mononuclear cells by examining the same HE staining specimen for each subject. The degree of distribution of these cells did not differ between the groups.

RT-PCR analysis of cytokine mRNA transcripts

Semiquantitative RT-PCR analysis showed that most of the inflamed sinus specimens exhibited constitutive expressions of GM-CSF, IL-5, IL-8, IL-16, eotaxin and

IFN- γ mRNA in various degrees, with amplification products seen at the predicted size. Expression levels of β -actin mRNA from each sample were found to be approximately the same. Figure 1 summarizes the degree of calculated relative ratios of these cytokines in the four groups categorized by the presence of AR and eosinophil accumulation. Among the four groups, we only found one group to be characteristic in terms of its cytokine expression profile. More specifically, the AR(-)Eo(-) group showed relative increases in IFN- γ mRNA expression and the difference was significant compared with the AR(-)Eo(+) and AR(+)Eo(+) groups. The AR(-)Eo(-) group also showed relatively low mRNA levels for GM-CSF, IL-5 and eotaxin. However, the other three groups appeared to show similar cytokine profiles, with high expression levels for GM-CSF, IL-5 and eotaxin mRNA and a low level of expression of IFN- γ mRNA. The expression levels for IL-8 and IL-16 mRNA did not differ between the four groups.

Immunohistochemical localization of CD4⁺ cell subsets

Because chemokine receptor expression at the site of inflammation can be a sensitive indicator of the type of local immune response, we examined, by immunofluorescence, CXCR3 and CCR4 expression on CD4⁺ cells infiltrating the sinus mucosa. Figure 2 shows a representative image of the distribution of CXCR3- and CCR4-positive cells in infiltrating CD4⁺ cells by double-immunofluorescent staining. This patient was from the AR(+)Eo(+) group and image analysis indicated that the mean density of eosinophils and CD4⁺ cells was 450 and 753.4 cells/mm², respectively. The CD4⁺ cells were scattered throughout the lamina propria and tended to cluster around blood vessels, with occasional

Table 1 Clinical background and histological characteristics of the study population

	Non-allergic patients (n = 77)		Allergic patients (n = 56)	
	Low eosinophils (n = 62)	High eosinophils (n = 15)	Low eosinophils (n = 41)	High eosinophils (n = 15)
Age (years)	48.8 $\pm$ 17	55.8 $\pm$ 15.4	36.4 $\pm$ 15.3*	46.8 $\pm$ 20.4
Sex ratio (male/female)	1.48	2.75	4.12	2
Peripheral blood eosinophils (%)	3.54 $\pm$ 2.9	6.84 $\pm$ 3.9*	3.69 $\pm$ 2.9	7.2 $\pm$ 3.2**
Mean tissue cell density (cells/mm ²)				
Eosinophils	36.3 (0–267)	655.3 (316.5–1610)	53.1 (0–200)	871.4 (310–2583)
Neutrophils	186.9 (0–983)	425.7 (33–2300)	295.1 (0–3200)	195.4 (0–733)
Mononuclear cells	1283.7 (16.7–6200)	1561.3 (217–3283)	2059.3 (50–7350)	1140.4 (242–4750)

Data are presented as either the mean $\pm$ SEM or the mean with the range given in parentheses. * $P < 0.05$, ** $P < 0.01$ compared with other groups.

cells detectable within the sinus epithelium. In this subject, more than 70% of CD4⁺ lymphocytes were found to be double positive for CCR4. In contrast, CXCR3-immunoreactive lymphocytes constituted less than 40% of the CD4⁺ cells. Hematoxylin and eosin staining of the consecutive specimen demonstrates infiltration of numerous eosinophils in the same area (Fig. 2e).

Figure 3 summarizes the density of CD4⁺ T cells and their subsets for each group analyzed by LSCM image systems. The results are expressed as the number of cells/mm². The total number of CD4⁺ cells was least in the AR(-)Eo(-) group (median 125.1 cells/mm²), with the decrease being significant compared with the other three groups (Fig. 3a). In both the allergic and non-allergic population, the density of CD4⁺ cells in the Eo(+) groups was significantly increased compared with that in the Eo(-) groups. In addition, baseline values of CCR4-positive CD4⁺ cells were significantly higher in the Eo(+) groups than in the Eo(-) groups, irrespective of the presence of AR (Fig. 3c). Interestingly, the absolute number of CD4⁺ T cells expressing CXCR3 also tended to increase in the Eo(+) groups. The density of CXCR3-positive cells in the AR(+)Eo(+) group was significantly higher than that in the AR(-)Eo(-) or AR(+)Eo(-) groups (Fig. 3b). As a result, the CXCR3⁺/CCR4⁺ cell ratio in the AR(-)Eo(-) group was 1.28 and significantly increased compared with the other three groups (Fig. 3d; AR(-)Eo(+), 0.53; AR(+)Eo(-), 0.65; AR(+)Eo(+), 0.63).

DISCUSSION

Various studies have implicated eosinophils as important players in the inflammation occurring in non-infectious chronic sinusitis.^{2,3,25} Whether or not the sinusitis is associated with AR, eosinophil infiltration seems to be a common parameter in the development of refractory chronic sinusitis.²⁶ In fact, recent reports have demonstrated that the levels of eosinophil infiltration are similar

between allergic and non-allergic patients in chronic sinusitis.^{11,27,28} Although several theories have been postulated as causes of the sinus tissue eosinophilia, the

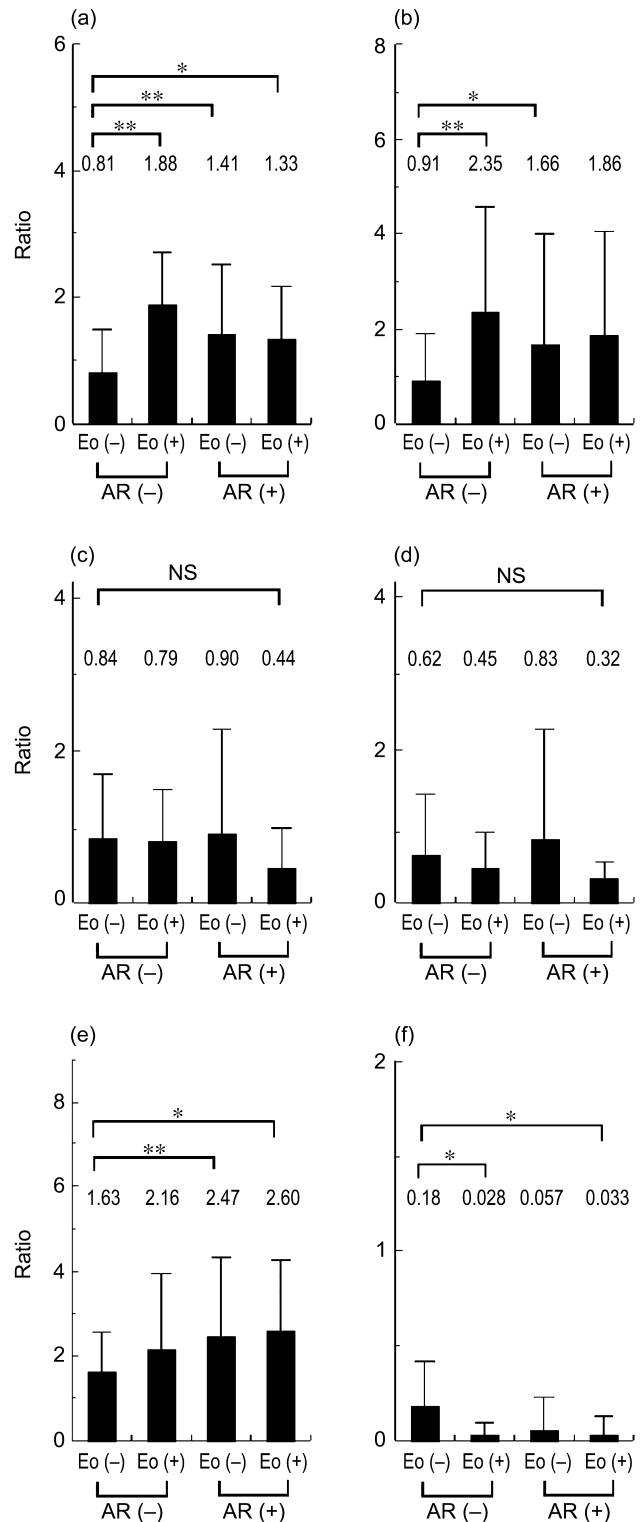


Fig. 1 Comparison of relative cytokine mRNA expression in sinus mucosal tissues between the four groups. The mRNA levels of (a) granulocyte-macrophage colony stimulating factor, (b) interleukin (IL)-5, (c) IL-8, (d) IL-16, (e) eotaxin and (f) interferon-γ (f) are expressed as a ratio relative to the expression of β-actin determined using reverse transcription-polymerase chain reaction. Data are the mean ± SEM of total specimens. **P* < 0.05, ***P* < 0.01. Eo(-), Eo(+), low and high eosinophilia, respectively; AR(+), AR(-), patients with and without allergic rhinitis, respectively.

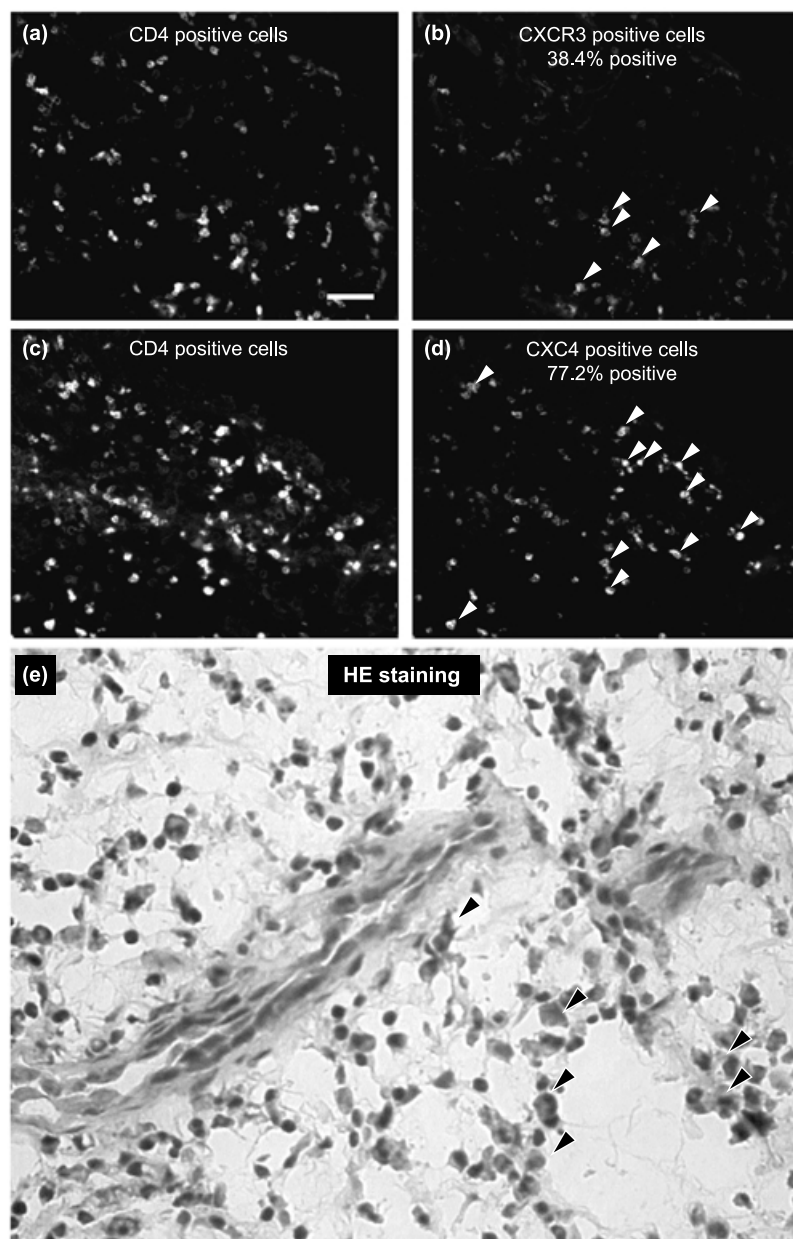


Fig. 2 Immunohistochemical detection of CXCR3 and CCR4 expression by CD4+ cells in the paranasal sinus mucosa. Cryostat sections of mucosal specimens from a representative subject in the AR(+)/Eo(+) group (allergic rhinitis with high eosinophilia). Double immunofluorescent staining with anti-CD4 (a) and anti-CXCR3 (b) antibodies in the same section revealed that 38.4% of CD4+ cells were found to be positive for CXCR3. In the consecutive section, double immunofluorescent staining with anti-CD4 (c) and anti-CCR4 (d) antibodies revealed that 77.2% of CD4+ cells were found to be positive for CCR4. Arrowheads indicate double-stained cells. Bar, 50 μ m. (e) Hematoxylin and eosin (HE) staining of the same specimen showing infiltration of numerous eosinophils in the same area (arrowheads).

original cause of eosinophil recruitment is not clear, especially in non-allergic subjects.

In the present study, patients were divided according to their dominant pathological features of eosinophilia. Although several studies have qualitatively and quantitatively provided data on the histopathologic features of chronic sinusitis, there are no standard levels of eosinophil density to define 'eosinophilic sinusitis'. For example, Berger *et al.* defined some sinusitis patients as polypoid mucosa and eosinophilia (PME) according to an arbitrary guideline based on gross microscopic

assessment.²⁵ They observed a significant increase in the number of eosinophils in the PME group compared with sinusitis patients with glandular hyperplasia (median value of 33.5 cells/mm²). In the present study, the distribution pattern of eosinophil density in all patients appeared to be a combination of two different standardized shapes, indicating the existence of two different sets of the sinusitis population. We consider that such classification constitutes a valuable contribution to the prevailing data on the pathology of chronic sinusitis among the Japanese population. Our findings also point towards

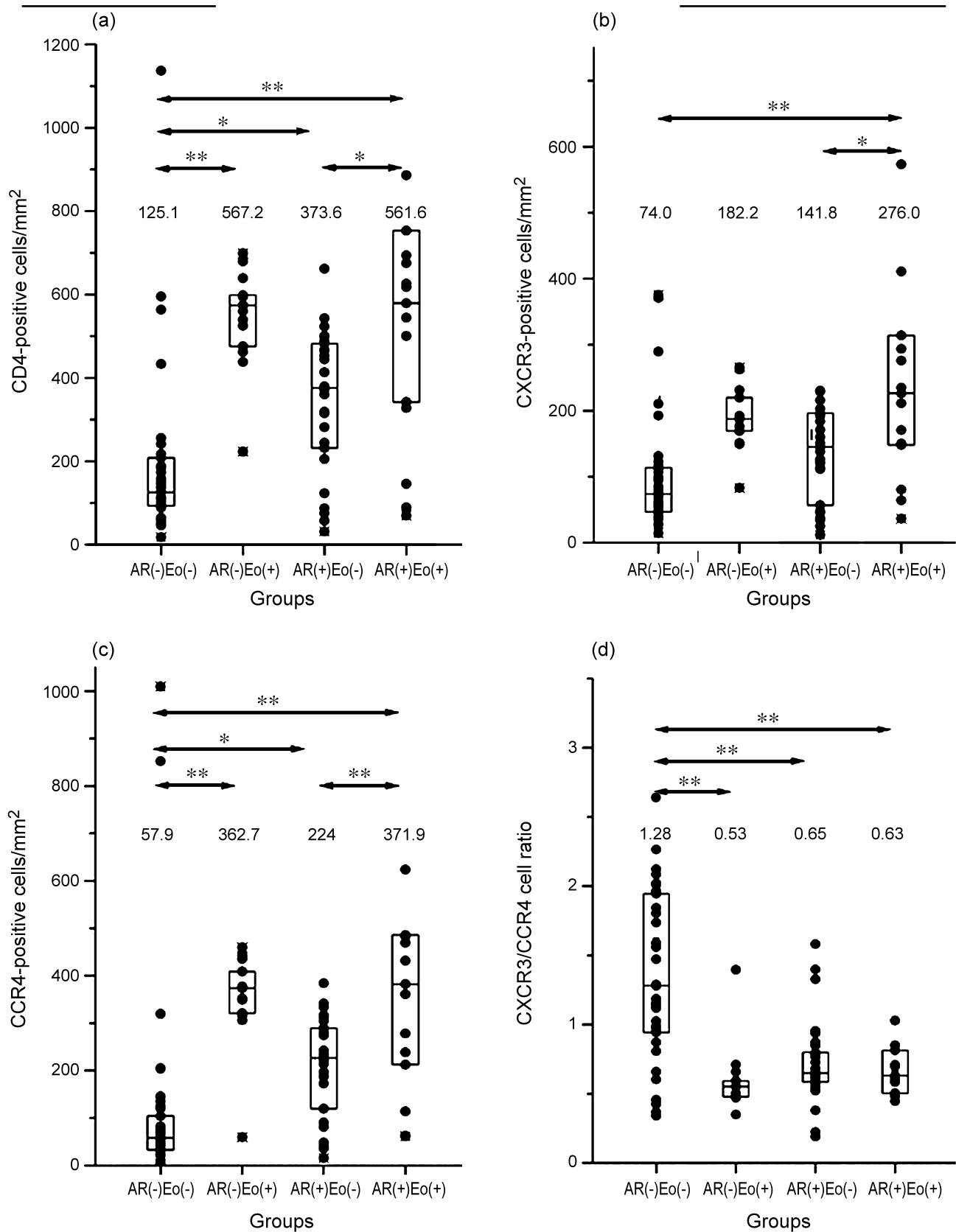


Fig. 3 Comparison of the density of (a) CD4-, (b) CXCR3- and (c) CCR4-expressing cells in the sinus mucosa between the four groups. Results are expressed as number of cells/mm². (d) Comparison of the CXCR3⁺/CCR4⁺ cell ratio between the four groups. The horizontal lines indicate the median value and the columns indicate the interquartile range for each group. *P < 0.05, **P < 0.01. Eo(-), Eo(+), low and high eosinophilia, respectively; AR(+), AR(-), patients with and without allergic rhinitis, respectively.

separate pathogenetic pathways that induce prolonged inflammatory responses and interference with normal mucociliary clearance.

Atopic diathesis can be considered to be a disorder of the whole respiratory tract, including sinus systems, with a broad spectrum of clinical features resulting from Th2 cytokine predominance.^{20–22,29,30} Hamilos *et al.*²¹ have reported that allergic and non-allergic subgroups show distinct cytokine profiles, with the most distinguishing cytokines of the allergic subgroup being IL-4 and IL-5, whereas those of the non-allergic subgroup are IFN- γ . Furthermore, patients with allergic sinusitis show an increased density of CD3⁺ T lymphocytes compared with either controls or patients with non-allergic sinusitis. Our findings also indicate that a Th2-dependent response is more evident in allergic patients during a chronic inflammatory event. The presence of both high levels of GM-CSF, IL-5 and eotaxin mRNA and low levels of IFN- γ mRNA in the AR(+) groups is consistent with previous reports. Interferon- γ is one of the Th1 cytokines and its production is depressed in CD4-positive cells from atopic subjects.³¹ Jyonouchi *et al.* analyzed the production of proinflammatory cytokines by sinus lavage cells and proposed that distinguished subsets of sinusitis patients without nasal polyposis existed on the basis of local IFN- γ production.³²

Furthermore, we observed a similar pattern of cytokine expression in non-allergic patients with high eosinophil infiltration. This finding is consistent with recent data obtained from both experimental animal models and humans, suggesting that there may be at least some pathophysiological consequences of allergic reactions even in the absence of IgE and mast cell responses.¹⁰ Jankovski *et al.*¹¹ examined the degree of eosinophil infiltration in a series of 263 adult patients with nasal polyposis. The number of eosinophils was increased in patients with asthma, although atopic patients did not have more eosinophils. These findings highlight evidence of the effects of Th2 cytokines underlying the development of chronic sinusitis with high eosinophil infiltration in certain non-allergic patients. Eosinophil accumulation in sinus tissue can also be attributable to an autostimulatory loop of related cytokines that may promote eosinophil survival regardless of atopic status.³³

The presence of CD4-positive lymphocytes within human airways appears to coordinate adaptive immune responses against foreign bodies and to promote inappropriate inflammatory cell infiltration. Within the airways of asthmatic subjects, CD4⁺ cells are considered to be

the major cellular sources of IL-4 mRNA, which is presumed to be critical to the development of Th2-type cells.³⁴ In addition, recent studies demonstrate that both activated T cells and eosinophils contribute to the induction of epithelial cell apoptosis.¹⁴ Activated effector T cells secrete IFN- γ and sensitize epithelial cells for tumor necrosis factor (TNF)- α -mediated apoptosis. In the present study, we found that a predominant number of CD4⁺ T cells and their subsets infiltrates the sinus mucosa concomitant with a high-density eosinophil infiltration, especially in patients with atopic diathesis. The density of CD4⁺ cells was significantly decreased in non-allergic patients with low eosinophilia. Furthermore, the density was significantly increased in the Eo(+) groups compared with the Eo(–) groups. These findings are consistent with previous reports by Driscoll *et al.*, who have found the presence of significantly more CD4⁺ cells in the sinus mucosa of sinusitis patients than in normal sphenoid sinus tissue, a finding that was not related to allergic status.³⁵ These data support the role of a CD4⁺ lymphocyte and Th2 cytokine axis in the pathogenesis of eosinophil inflammation and subsequent tissue damage in the sinus mucosa.

The balance between Th1 and Th2 responses is thought to be important for the outcome of immune reactions and disease courses. A series of recent *in vitro* studies has demonstrated that Th1 and Th2 cells express distinct sets of chemokine receptors that may regulate the recruitment and localization of these cells to inflammatory sites.^{15–17} Cells expressing CXCR3 and CCR5 have been associated with the Th1 cytokine profile,¹⁸ whereas CCR3, CCR4 and CCR8 have been associated with the Th2 phenotype.¹⁹ Previous reports largely indicate that CCR4 and, to a lesser extent, CCR8 mark the majority of T cells infiltrating the airways of atopic patients. For example, Panina-Bordignon *et al.*³⁶ have demonstrated that the majority (>90%) of T cells infiltrating the bronchial biopsies of allergen-challenged asthmatics produce IL-4 and express CCR4. In addition, Kurashima *et al.*³⁷ have measured CXCR3- and CCR4-expressing CD4⁺ T cells by flow cytometry in patients with asthma. They found that, in the steroid-treated asthma group, there was a decrease in CCR4⁺ T cells and an increase in the CXCR3⁺/CCR4⁺ ratio, suggesting that oral corticosteroids modulate the balances of CXCR3⁺ and CCR4⁺ cells. In the present study, CCR4-expressing CD4⁺ cells in the sinus mucosa were significantly higher in patients with high eosinophilia, both in allergic and non-allergic populations. The CXCR3⁺/CCR4⁺ cell ratio in these

patients was significantly decreased compared with that in non-allergic patients with low eosinophilia. These data suggest that a shift to a more type 2-dominated response may occur in the sinus mucosa of allergic patients and some non-allergic patients when they are exposed to unknown external stimuli. The contribution of selective induction of CD4⁺ effector T cell subsets to the development of different pathological conditions has also been reported previously in patients with seasonal allergic rhinitis.³⁸

The exact reason for a shift to a more type 2-dominated response in the sinus mucosa of non-allergic patients with high eosinophil infiltration is still unclear. However, it seems that more than one mechanisms may contribute to the Th2 dominance and persistent eosinophil infiltration in the sinus mucosa. Terada *et al.*³⁹ mentioned that a hypoxic state in paranasal sinuses activates endothelial cells and fibroblasts to release inflammatory mediators, leading to promotion of the adherence of inflammatory cells to the endothelium. These reaction systems include proinflammatory cytokines, such as TNF- α and IFN- γ , as well as Th2 cytokines, including IL-4 and IL-13, even in non-atopic subjects. Furthermore, we previously observed constitutive activation of nuclear factor (NF)- κ B, a transcription factor deeply implicated in the regulation of various inflammatory cytokines, in the nasal polyp epithelium.²³ A close correlation existed between the degree of NF- κ B activation and the levels of IL-8, IL-16 and eotaxin mRNA expression. The increased NF- κ B activity in the epithelium can be considered to reflect hypersensitivity to external stimuli and to be responsible, in part, for the recruitment of eosinophils.

Until now, very limited information has been available on the expression and function of specific chemokine receptors during the inflammatory response in the sinus mucosa. We do realize that cross-sectional analysis is less powerful in revealing differences in cytokine or chemokine receptor expression between atopic and non-atopic subjects compared with prospective longitudinal analysis. Relatively large interindividual differences in cytokine mRNA and chemokine receptor expression for each group may partly reflect variation in age, time of surgery or the period for which the disease has been suffered. However, the present study has, for the first time, investigated the expression of CXCR3 and CCR4 in sinusitis patients categorized by allergic status and eosinophil infiltration. The finding that a repertoire of immune responses similar to atopic patients can also be observed in patients in the AR(-)Eo(+) group provides support for

the concept of chronic sinusitis as a Th2-mediated disease process. In addition, a mixture of Th1 and Th2 type lymphocytes seems to orchestrate the inflammatory response that leads to various final pathologic profiles, including intense eosinophil infiltration.

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