
Expression of membrane-bound CD23 in nasal mucosal B cells from patients with perennial allergic rhinitis

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Background: CD23 is the low-affinity receptor for IgE on B cells and is thought to play an important role in regulation of IgE production.

Objective: To measure the expression of membrane-bound CD23 in nasal B cells and examine its correlation with CD4 subtypes or serum IgE levels in patients with perennial allergic rhinitis.

Method: We used flow cytometric analysis with double, direct immunofluorescence staining of the mucosal-infiltrating lymphocytes to examine the expression of CD23 in nasal mucosal B cells of patients with perennial allergic rhinitis. The expression of CD23 in nasal B cells of patients with nonatopic rhinosinusitis served as a control.

Result: The ratio of CD23⁺ B cells to total B cells in patients with perennial allergic rhinitis was significantly higher than in nonatopic controls, whereas that of B cells to total lymphocytes was unchanged. The ratio of CCR4⁺ CD4 cells to total CD4 cells in allergic patients was significantly higher than in nonatopic controls, whereas the ratio of CXCR3⁺ CD4 cells to total CD4 cells was unchanged. There was no significant correlation between the percentages of CD23⁺ B cells and CCR4⁺ CD4 cells. In addition, the percentage of CD23⁺ B cells did not correlate with the total IgE level or with the specific IgE level.

Conclusions: Our results indicate that nasal mucosal CD23-bearing B cells, as well as T_H2 cells, increase in patients with perennial allergic rhinitis. However, the expression of CD23 did not directly correlate with the number of T_H2 cells in the nasal mucosa.

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INTRODUCTION

Allergic rhinitis (AR) occurs through fundamental mechanisms that involve induction of allergen-specific IgE antibodies. Allergen-specific T-cell–B-cell interactions are indispensable for the induction of human IgE synthesis, and it has recently been reported that interleukin 4 (IL-4) and other cytokines released from CD4 helper cells (T_H2 cells) affect T-cell–B-cell interactions and play a role in the induction of IgE synthesis in B cells.^{1,2}

Human CD23 exists in 2 isoforms (CD23a and CD23b), which differ only in 6 or 7 amino acids at the N terminus. CD23 has the potential to associate with HLA-DR at the surface of B cells and in doing so may help to stabilize T-cell–B-cell interactions, which in turn contribute to T-cell activation.³ The membrane-bound CD23 on B cells is thought to enhance IgE-dependent antigen presentation to T cells and also to influence IgE synthesis in the B cells. However, CD23 expression on B cells in the nasal mucosa and its possible correlation with relevant T_H2 cells in patients with allergic diseases have yet to be clarified. In the present study, we measured the expression of membrane-bound CD23 in nasal

B cells and examined its correlation with CD4 subtypes or serum IgE levels in patients with perennial allergic rhinitis.

MATERIALS AND METHODS

Patients

Japanese patients with serious perennial AR due to *Dermatophagoides pteronyssinus* were enrolled in this study. The diagnosis of AR was made based on the criteria of Okuda et al,⁴ including a positive CAP radioallergosorbent test result (greater than class 2; SRL, Tokyo, Japan) against *D pteronyssinus*. None of the patients received immunotherapy or immunosuppressive drugs (including steroids) during the study. Japanese patients with nonatopic rhinosinusitis were enrolled as controls. Informed consent for participation in the study was obtained from each participant.

Tissue Samples

Inferior turbinate mucosa or paranasal mucosa was obtained by endonasal sinus surgery. After the mucosa was cut into small pieces (approximately 2 mm), tissue-infiltrating lymphocytes were collected with a cell strainer (Falcon, Discovery Labware, BD Biosciences, Bedford, MA), using the Ficoll-Hypaque separation technique (lymphocyte separation solution, Nacalai Tesque Inc, Tokyo, Japan). The tissue-infiltrating lymphocytes were washed twice with phosphate-buffered saline (PBS) and resuspended in a freezing solution

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(Cell Banker, Nihon Zenyaku, Fukushima, Japan). The cells were stored at -80°C until examination.

Antibodies

Anti-human CD4, CD19, CD23, and CXCR3 monoclonal antibodies were purchased from Dako Corporation (Tokyo, Japan). Anti-human CCR4 monoclonal antibody was obtained from Genzyme (Boston, MA).

Flow Cytometric Analysis

The frozen cells were rapidly thawed and diluted 10 times with PBS that contained 1% bovine serum albumin (BSA). After 2 washes with PBS in 1% BSA, the cells were stained with an fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-CD19 antibody combined with a R-phycoerythrin (RPE)-conjugated anti-CD23 antibody or with a FITC- or RPE-conjugated negative control antibody, according to the manufacturer's protocol. The cells were also stained with an FITC-conjugated anti-CD4 antibody combined with RPE-conjugated anti-CXCR3 or anti-CCR4 antibodies.

Cells were subjected to flow cytometric analysis using a flow cytometer (FACScan, Becton, Dickinson and Company, Franklin Lakes, NJ). A lymphocyte gate was set based on the pattern of forward and side scatter. A minimum of 5×10^4 cells in the gate was analyzed on the same day. B lymphocytes were identified as CD19⁺ lymphocytes, and T_H cells were identified as CD4⁺ lymphocytes. Cell viability was demonstrated by negative staining with 7-aminoactinomycin D (Sigma-Aldrich, St Louis, MO), which showed that at least 98% of the cells were viable.

Statistical Analysis

Statistical analysis was performed using a Wilcoxon rank sum test or a Wilcoxon signed rank test for paired and unpaired data. Statistical analysis was also performed using a Spearman rank correlation test for correlation between the data. $P < .05$ was considered statistically significant. Data are presented as mean $\pm$ SD.

RESULTS

Patients

Eleven Japanese patients (mean $\pm$ SD age, 41.1 ± 18.7 years; age range, 23–69 years; 5 men and 6 women) with serious perennial AR due to *Dermatophagoides pteronyssinus* were enrolled in the study as study patients. Eleven Japanese patients (mean $\pm$ SD age, 50.4 ± 14.3 years; age range, 24–71 years old; 7 men and 4 women) with nonatopic rhinosinusitis were enrolled as controls.

Dot Plots for CD19 FITC and CD23 RPE

Typical dot plots for CD19 FITC and CD23 RPE staining are shown for the control group and the AR group in Figure 1. Only CD19⁺ cells expressed CD23 on mucosal lymphocytes, and CD23 expression on B cells from AR mucosa was higher than that of controls. The dot plot pattern of CD23 expression on nasal B cells suggested that this was not an all or nothing effect for a given cell but rather that B cells expressed various levels of CD23. Therefore, we measured the percent positive and mean fluorescence intensity (MFI) of CD23 on B cells, where the percent positive value indicates the relative amount

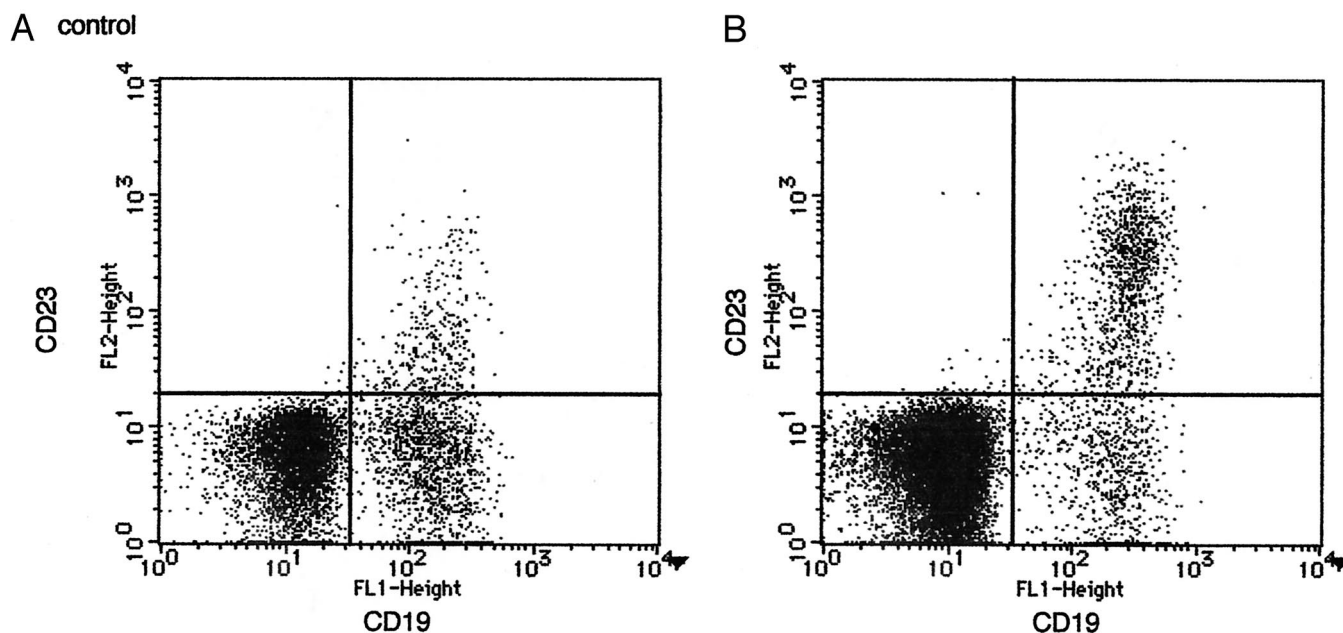


Figure 1. Representative dot plots for fluorescein isothiocyanate-conjugated anti-CD19 (FL1) and R-phycoerythrin-conjugated anti-CD23 (FL2) antibodies for the control group (A) and the allergic rhinitis (AR) group (B). The proportion of CD23-bearing B cells was measured by flow cytometry as CD19 and CD23 double-positive plots.

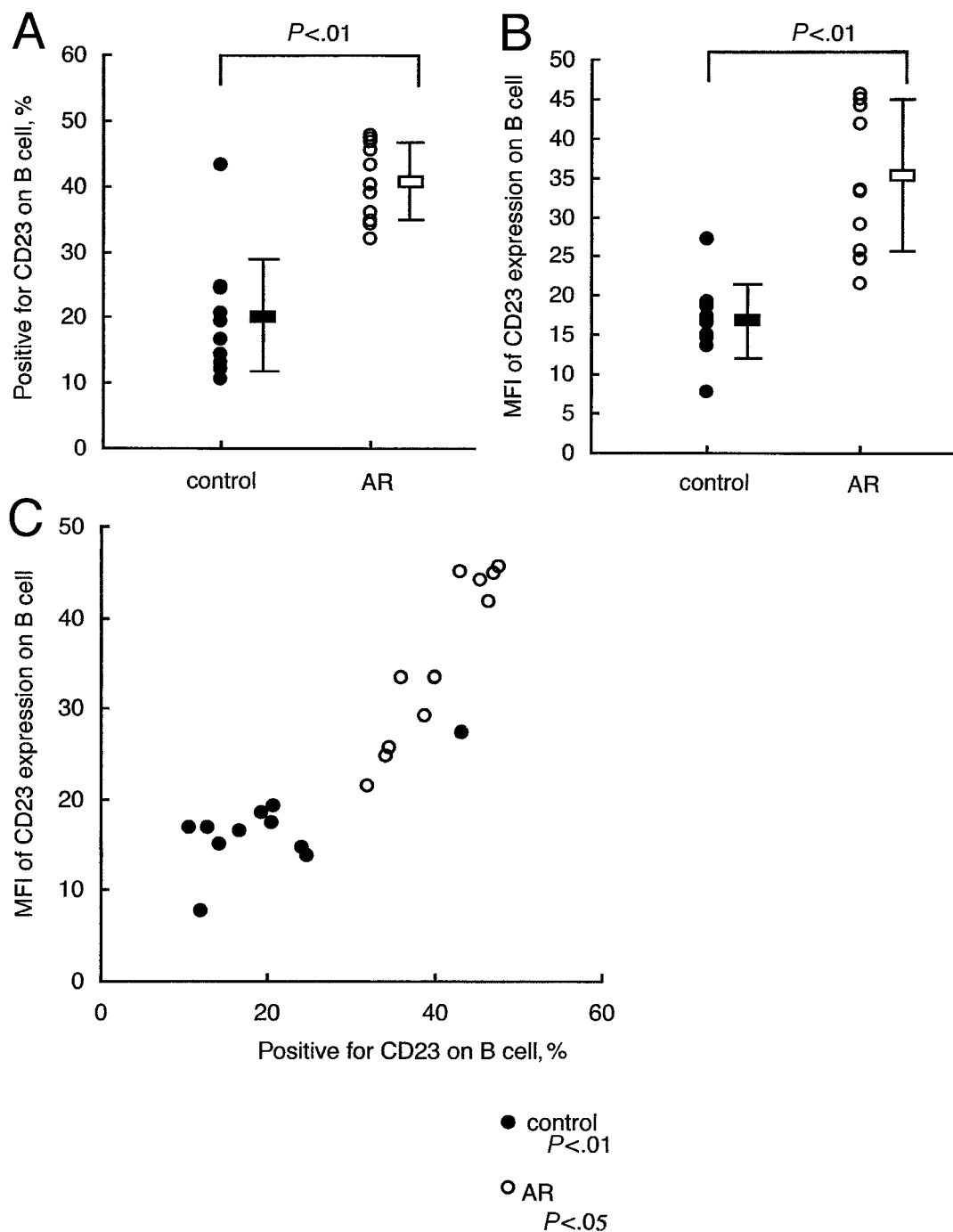


Figure 2. CD23 expression on B cells. A, Percent positive values for CD23 on B cells from patients with allergic rhinitis (AR) were significantly higher than for the control group. B, A similar tendency was seen in the mean fluorescence intensity (MFI) of CD23 expression on B cells, which was significantly increased for patients with AR compared with controls. C, Correlation plot between percent positive values for CD23 on B cells and MFI of CD23 expression on B cells. The percent positive values for CD23 on B cells was significantly correlated with the MFI of CD23 expression on B cells for the control group and the AR group.

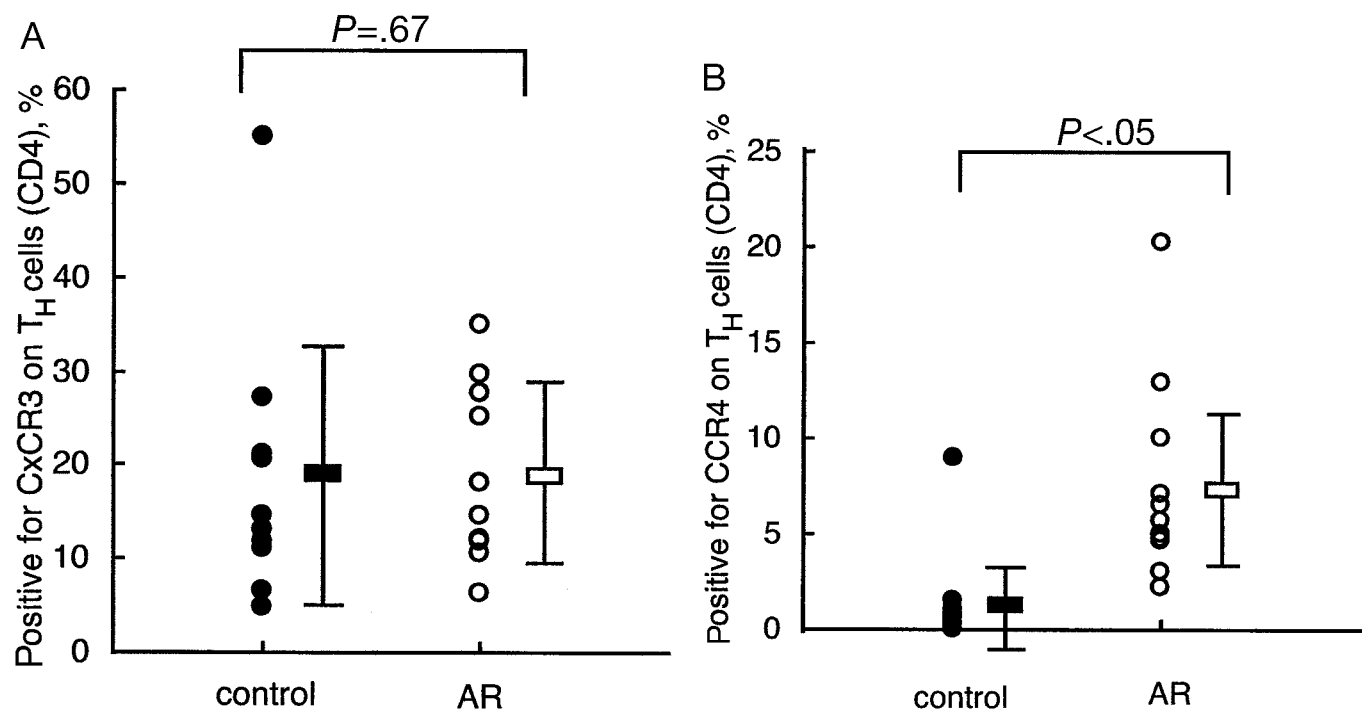


Figure 3. Percent positive values for chemokine receptor expression on mucosal CD4 T cells. A, Data for the CXCR3 subtype, assumed to be T_H1 cells. B, Data for the CCR4 subtype, assumed to be T_H2 cells. There is no significant difference in percent positive values for CXCR3 on CD4 cells from patients with allergic rhinitis (AR) and controls (A), whereas the percent positive values for CCR4 on mucosal CD4 cells from patients with AR were significantly higher than in controls.

of CD23⁺ B cells to total B cells, and the MFI indicates the mean level of CD23 expression per B cell.

Expression of CD23 on Mucosal B Cells

The percent positive value for CD23 on mucosal B cells in the AR group ($43.9\% \pm 5.8\%$) was significantly higher than in the control group ($19.9\% \pm 9.0\%$, $P < .001$) (Fig 2A), whereas that of B cells to total lymphocytes was unchanged (data not shown). The MFI of CD23 on mucosal B cells in the AR group (40.76 ± 20.62) was also significantly higher than in the control group (16.9 ± 4.68 , $P = .004$) (Fig 2B). The percent positive value and the MFI for CD23 were significantly correlated, with the correlation coefficients for control subjects and AR patients being 0.71 ($P = .03$) and 0.99 ($P = .008$), respectively (Fig 2C).

Expression of Chemokine Receptors on Mucosal CD4 Cells

CXCR3 and CCR4 were used as T_H1 and T_H2 markers, respectively.⁵ There was no difference between the percent positive value for CXCR3 on mucosal CD4 cells in the AR group ($16.3\% \pm 8.1\%$) and in the control group ($18.8\% \pm 13.8\%$, $P = .67$) (Fig 3A), whereas the percent positive value for CCR4 on mucosal CD4 cells in the AR group ($7.5\% \pm 5.7\%$) was significantly higher than in the control group ($1.3\% \pm 2.5\%$, $P = .02$) (Fig 3B).

Correlation Between Percent Positive Values for CD23 on B Cells and CCR4 on CD4 Cells

No significant correlation was observed between the percent positive values for CD23 on mucosal B cells and CCR4 on CD4 cells from the same mucosa. The correlation coefficients for control subjects and AR patients were 0.21 ($P = .25$) and 0.26 ($P = .17$), respectively (Fig 4); hence, there was no significant correlation in either group.

Correlation Between the Percent Positive Value for CD23 on B Cells and the Serum IgE Level

The correlation between the percent positive values for CD23 on mucosal B cells and total serum IgE levels is shown in Figure 5. No significant correlation was observed between these values or between the percent positive values for CD23 on B cells and specific IgE levels (data not shown).

DISCUSSION

The role of CD23 in IgE synthesis is still controversial and remains to be elucidated. The binding of the antigen-IgE complex to CD23-bearing B cells has been shown to augment IgE-mediated responses.⁶ In addition, CD23 is the enhancement of IgE-dependent antigen presentation to T cells.^{3,7,8} In clinical studies, the cell surface expression in peripheral blood B lymphocytes has shown increased CD23 expression

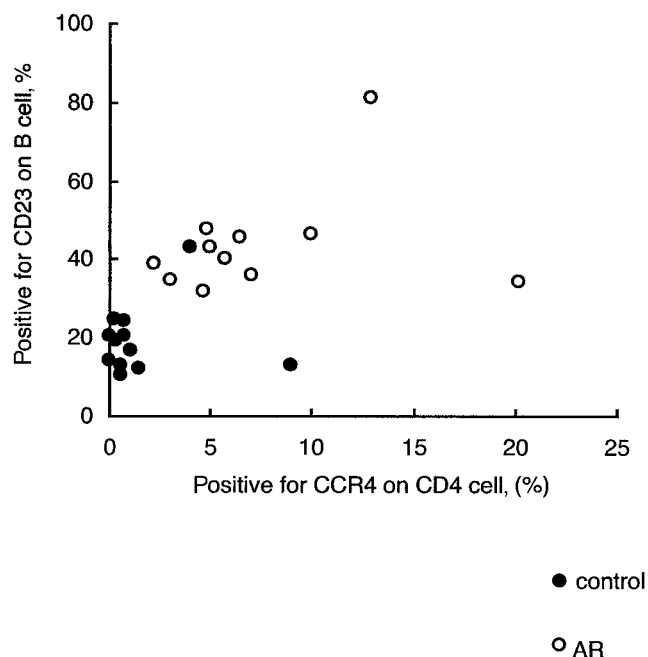


Figure 4. Correlation plot for percent positive values for CD23 on mucosal B cells and percent positive values for CCR4 on mucosal CD4 cells. There is no significant correlation between these data in the control group ($P = .25$) or the allergic rhinitis (AR) group ($P = .17$).

in allergic children and adults, including patients with AR compared with nonallergic controls,^{9,10} and has further shown that CD23 expression decreased after successful hyposensitization.^{11,12} Furthermore, since IgE levels in serum were evaluated as an atopy marker, significant correlations were

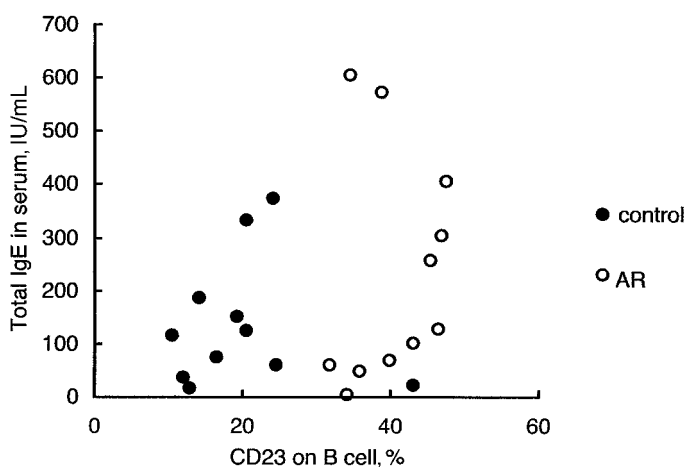


Figure 5. Correlation plot for percent positive values for CD23 on mucosal B cells and the total IgE level. There is no significant correlation between these data in the control group ($P = .21$) or the allergic rhinitis (AR) group ($P = .66$).

reported between the levels of the soluble form of CD23 and the levels of IgE in serum in patients with atopy.¹³

In the present study, we examined the expression of CD23 on mucosal B cells and found higher percentages of CD23-bearing B cells in patients with perennial AR compared with those in nonallergic patients. In addition, we investigated T_H1 and T_H2 cells in the nasal mucosa by staining for expression of CXCR3 and CCR4 chemokine receptors, respectively. The results showed that the $T_H2/CD4$ ratio in patients with perennial AR was indeed higher than in nonallergic controls, whereas the $T_H1/CD4$ ratio was unchanged. However, no significant correlation was found between the $T_H2/CD4$ ratio and the CD23/B-cell ratio. Furthermore, no significant correlation was found between the CD23/B-cell ratio in the nasal mucosa and the total IgE level or specific IgE level in serum (data not shown).

The T-cell–B-cell interaction must play an important role in allergic inflammation. IL-4 and IL-13 are known to promote the switching of B cells from IgM to IgE production and expression of CD23,¹⁴ whereas interferon- γ , IL-10, and IL-12 inhibit this effect.^{15–17} Other than T_H2 cells, various kinds of cells in the nasal mucosa, such as mast cells, basophils, and CD8 cells, have been shown to produce IL-4 and IL-13.^{18,19} The lack of a significant correlation between the ratio of $T_H2/CD4$ T cells with CD23/B cells may suggest that the total amount of IL-4 and/or IL-13 produced from not only T_H2 cells but other cells influences CD23 expression in nasal mucosal B cells. In this study, T_H2 cells were shown to make up approximately 7.15% of the infiltrating CD4 T cells in the nasal mucosa of patients with AR. However, only a small portion of these T_H2 cells could recognize the house dust mite allergen. An enzyme-linked immunosorbent spot-forming cell assay study has shown a low frequency population of allergen-specific IL-4- or IL-13-producing T_H cells, which represented approximately 1 spot per 10,000 to 100,000 peripheral CD4 T cells.^{20,21} T_H1/T_H2 cytokine dysregulation is thought to be a fundamental pathogenesis of AR, but only a few T and B cells are allergen specific. The major source of IL-4 and IL-13 production in the effector phase in the nasal mucosa of patients with AR may be mast cells or basophils and not T_H2 cells.²² The role of T_H2 cytokines from mast cells remains to be clarified, but a recent study showed that T_H2 cytokines from mast cells are induced by antigen stimulation²³ and influence not only the differentiation of naive T cells toward T_H2 cells²⁴ but also B-cell activation.²⁵ In addition, the lack of correlation between the number of nasal B cells and the serum IgE level observed in this study may suggest that the nasal mucosa could synthesize IgE independently from peripheral blood.²⁶

Overall, the results of this study suggest that enhanced expression of CD23 on nasal mucosal B cells occurs in patients with AR. However, further analysis is required regarding the significance of CD23 in nasal mucosa at the site of the allergic reaction.

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