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.

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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

(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 ± SD.

RESULTS

Patients

Eleven Japanese patients (mean ± 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 ± 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

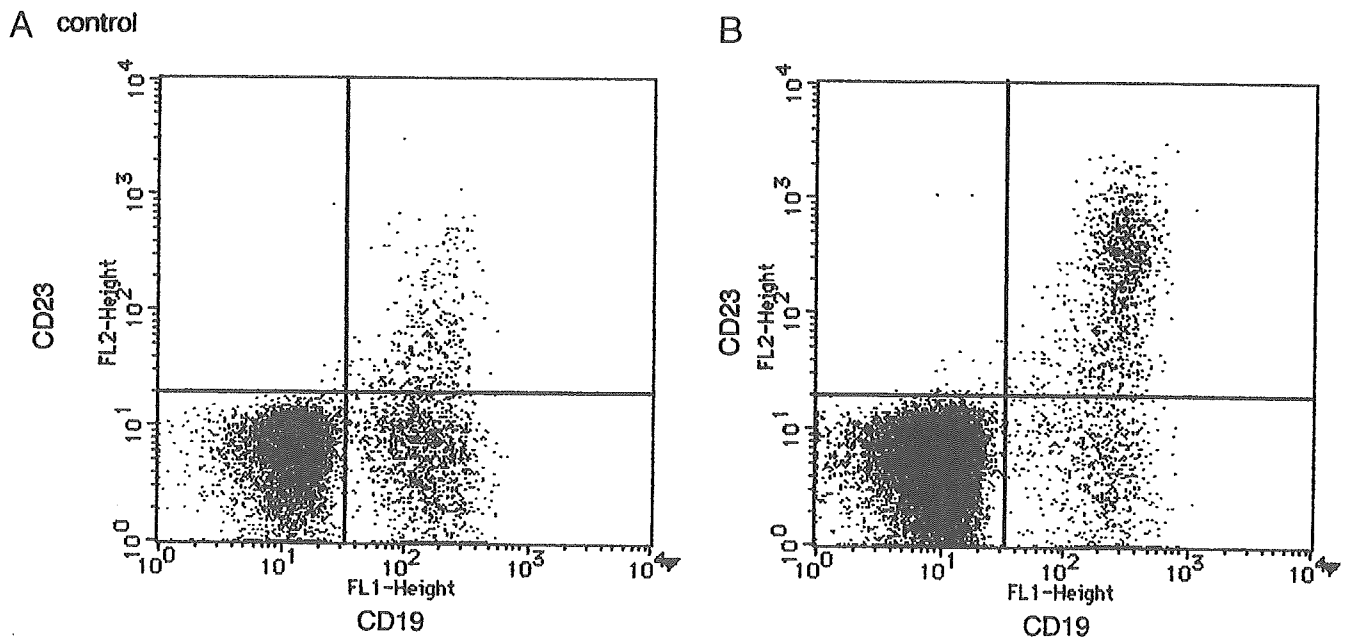

Figure 1. Representative dot plots for fluorescein isothiocyanate–conjugated anti-CD19 (FL1) and R-phycoerythrin–conjugated anti-CD23 (FL2) antibodies for the control group (A) and the allergic rhinitis (AR) group (B). The proportion of CD23-bearing B cells was measured by flow cytometry as CD19 and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

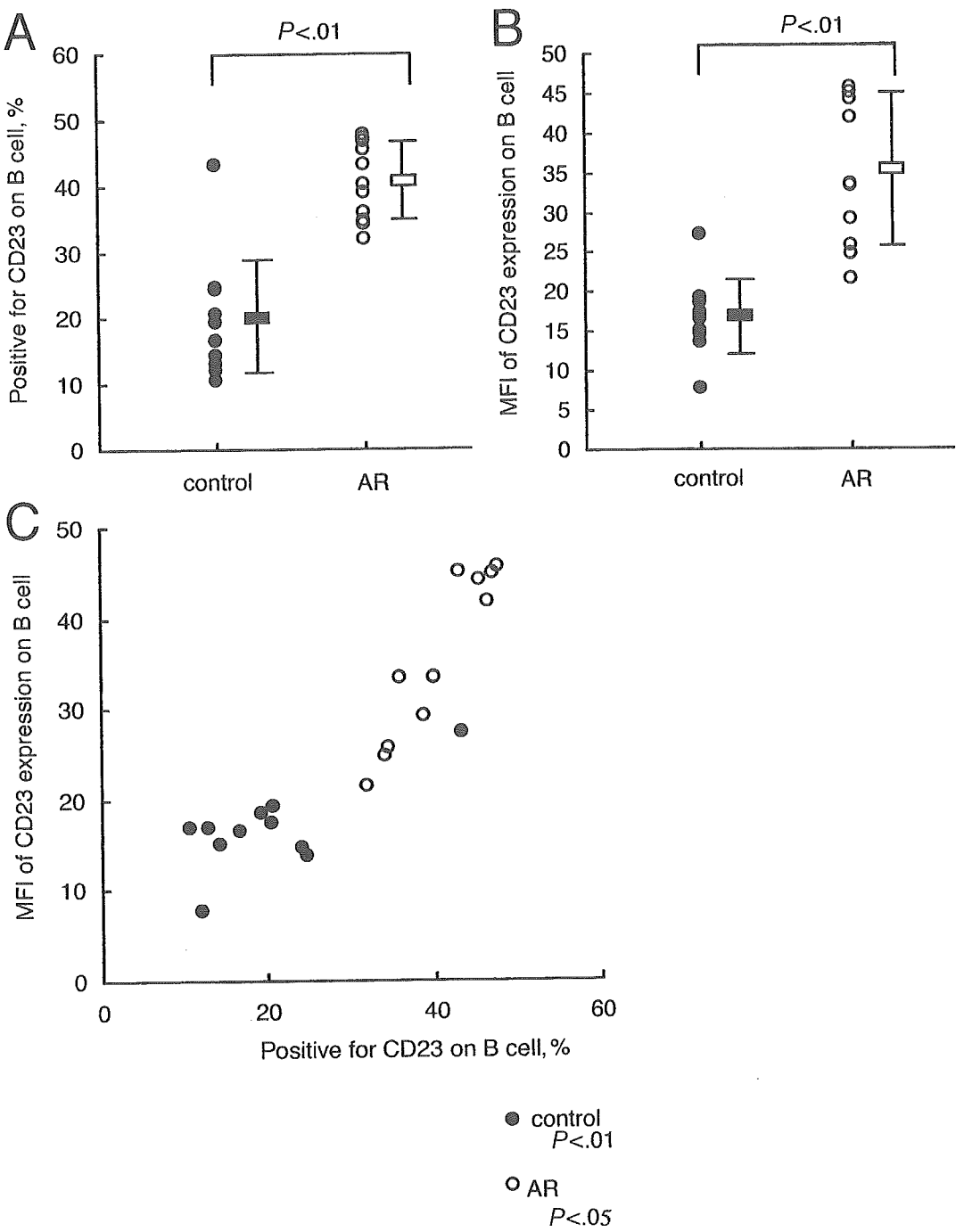

Figure 2. CD23 expression on B cells. A, Percent positive values for CD23 on B cells from patients with allergic rhinitis (AR) were significantly higher than for the control group. B, A similar tendency was seen in the mean fluorescence intensity (MFI) of CD23 expression on B cells, which was significantly 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.

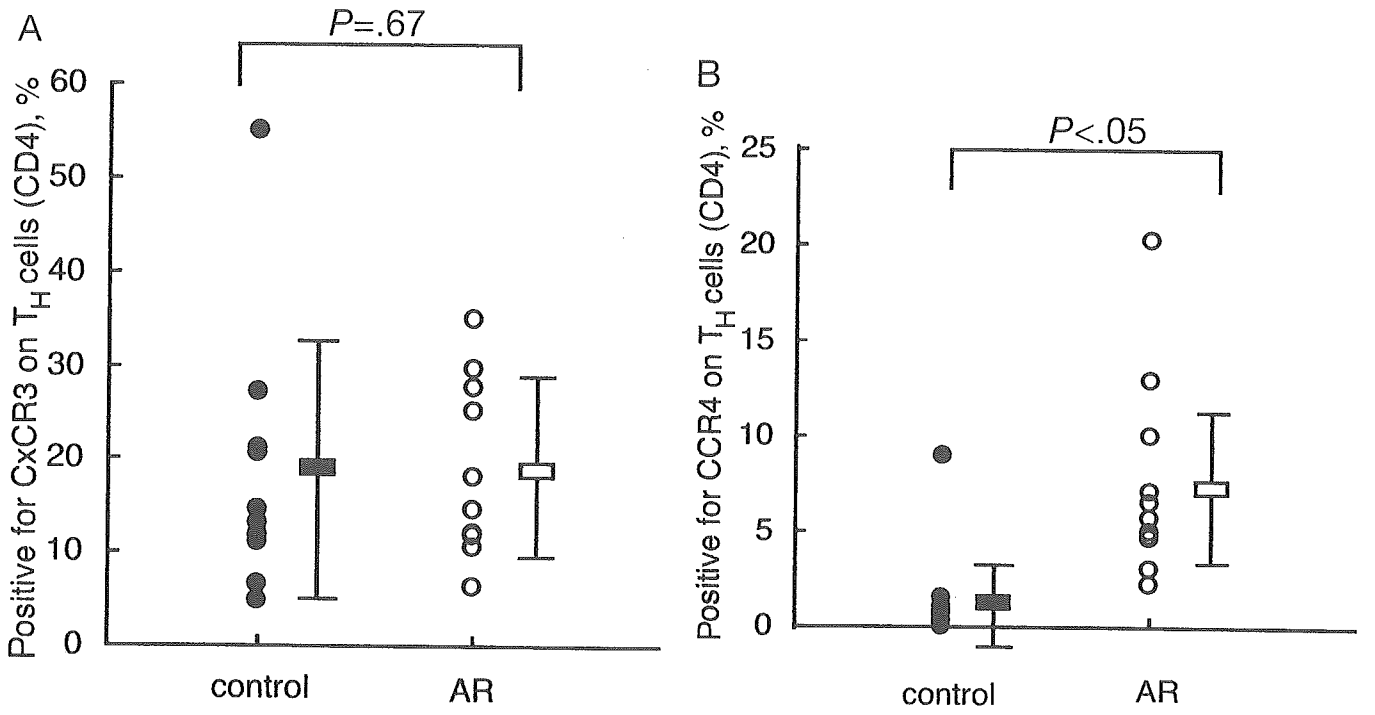

Figure 3. Percent positive values for chemokine receptor expression on mucosal CD4 T cells. A, Data for the CXCR3 subtype, assumed to be T_H1 cells. B, Data for the CCR4 subtype, assumed to be T_H2 cells. There is no significant difference in percent positive values for CXCR3 on CD4 cells from patients 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% ± 5.8%) was significantly higher than in the control group (19.9% ± 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% ± 8.1%) and in the control group (18.8% ± 13.8%, $P = .67$) (Fig 3A), whereas the percent positive value for CCR4 on mucosal CD4 cells in the AR group (7.5% ± 5.7%) was significantly higher than in the control group (1.3% ± 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

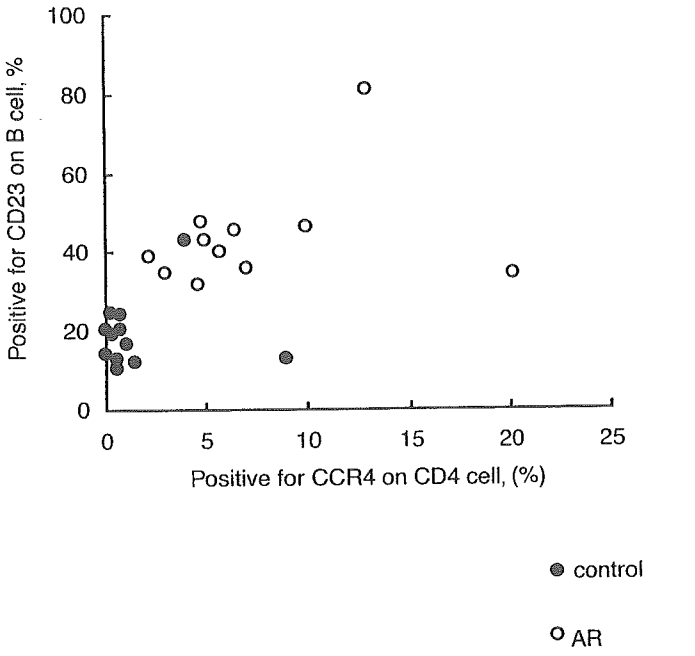

Figure 4. Correlation plot for percent positive values for CD23 on mucosal B cells and percent positive values for CCR4 on mucosal CD4 cells. There is no significant correlation between these data in the control group (P = .25) or the allergic rhinitis (AR) group (P = .17).

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

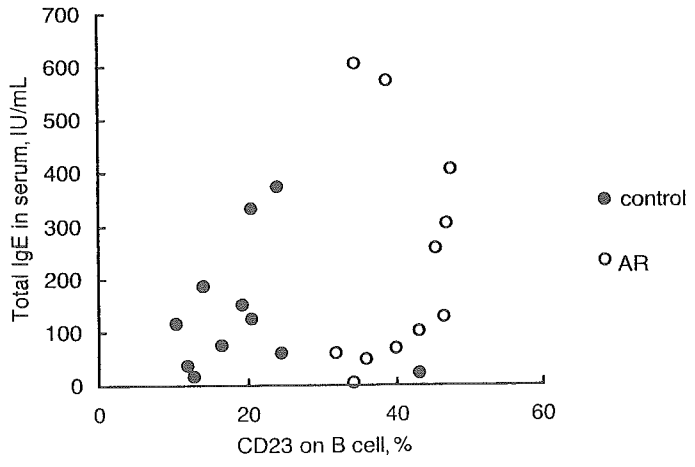

Figure 5. Correlation plot for percent positive values for CD23 on mucosal B cells and the total IgE level. There is no significant correlation between these data in the control group (P = .21) or the allergic rhinitis (AR) group (P = .66).

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 roll 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 stimulation[23] and influence not only the differentiation of naive T cells toward T_H2 cells[24] 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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Role of T cells in allergic rhinitis

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Summary

A characteristic feature of allergic rhinitis (AR) is the production of allergen-specific IgE antibodies. It is widely accepted that the induction of antigen-specific type 2 $CD4^+$ helper cells (Th2) is a key event in this disease. Allergen-specific T cell–B cell interaction is indispensable for the induction of human IgE synthesis. Th2 cells also cause influx and activation of eosinophils, mast cells and basophils. Th2 cytokine-inducing cells play a major role in AR; this is known as the 'Th2 paradigm'. In this review, we examine the Th1/Th2 cell dysregulation of AR and discuss new treatment approaches to improve this dysregulation.

Keywords acquired immunity, allergic rhinitis, innate immunity, T cells, Th1 cell, Th2 cell

Allergic rhinitis and T cells

Increasing numbers of patients with allergic rhinitis (AR) are being seen on a global scale [1]. Although over 90% of patients with perennial AR exhibit allergic reactions to mite antigen, the major allergens in Japan are Japanese cedar and Japanese cypress pollens [1]. These pollens can spread more than 100 km from the tree and are, therefore, able to affect distant metropolitan areas. Japan's situation is hence distinct from that in European countries, where the most common pollinosis allergen (ragweed) can only disperse over much shorter distances of up to several hundred metres.

Symptoms of AR arise as a result of induction of allergen-specific IgE antibodies [2]. The progression of AR may be divided into immediate- and late-phase disease. Sneezing, runny nose and nasal obstruction are observed during the immediate-phase response, in which specific IgE antibody-bearing mast cells are thought to play a central role. These mast cells release various chemical mediators such as histamine, leukotrienes and prostaglandins that are causative of nasal symptoms. In contrast, the late-phase response manifests 6–8 h after allergen exposure and has a different mechanism that is characterized by recruitment and activation of eosinophils, basophils and activated T cells at the sites of allergen exposure.

It is widely accepted that induction of antigen-specific type 2 $CD4^+$ helper cells (Th2) is a key event in many allergic diseases including AR. Allergen-specific T cell–B cell interaction is indispensable for the induction of human IgE synthesis. IL-4 and other Th2 cytokines are believed to affect T cell–B cell interactions and induction of IgE synthesis from B cells [3, 4]. These Th2 cells also cause influx and activation of eosinophils, mast cells and basophils, a phenomenon known as the 'Th2 paradigm'. Although Th2 cells may have a causal role in AR, some aspects of this involvement are unclear. For example, production of allergen-mediated IFN-γ in peripheral blood mononuclear cells from AR patients is comparable with that in cells obtained from non-allergic control subjects. IFN-γ, secreted by Th1 cells, inhibits Th2 cells. Moreover, the reversible Th1 ← → Th2 hypothesis has not been reliably demonstrated *in vivo* even in successful specific immunotherapy. This suggests that Th1/Th2 dysregulation is not the cause of nasal allergy [5, 6]. Th1/Th2 cellular dysregulation in AR is yet to be clarified.

We investigated the T cell subpopulation in nasal mucosa samples from patients with AR vs. control subjects. Tissue-infiltrating lymphocytes from nasal mucosa were stained with FITC-conjugated CD4 combined with RPE-conjugated anti-CXCR3 or CCR4 monoclonal antibodies (used as Th1 and Th2 markers, respectively) and subjected to flow cytometric analysis. The percentage of mucosal $CD4^+$ cells positive for CCR4 was higher in patients than in non-allergic controls, while there was no significant difference between the percentage of mucosal $CD4^+$ cells positive for CXCR3 between the two groups (Fig. 1), suggesting that the level of Th1 in patients with AR is similar to that in non-allergic subjects. We further examined the Th1/Th2 profile in peripheral blood $CD4^+$ T cells from allergic and non-allergic subjects using the same approach [7]. As shown in Fig. 2, there was no significant difference in the number of Th1/Th2 cells in peripheral blood $CD4^+$ T cell populations between the two groups, suggesting that Th1/Th2 cellular dysregulation is not evident in peripheral blood.

Recently, we attempted direct detection of specific Th1/Th2 cell numbers in AR by major histocompatibility complex (MHC) class II epitope-mediated enzyme-linked immunospot (ELISPOT) method, and found very low population numbers of allergen-specific IL-4-producing Th cells (which represented approximately 1 spot/10^4–10^5 $CD4^+$ T cells). Interest-

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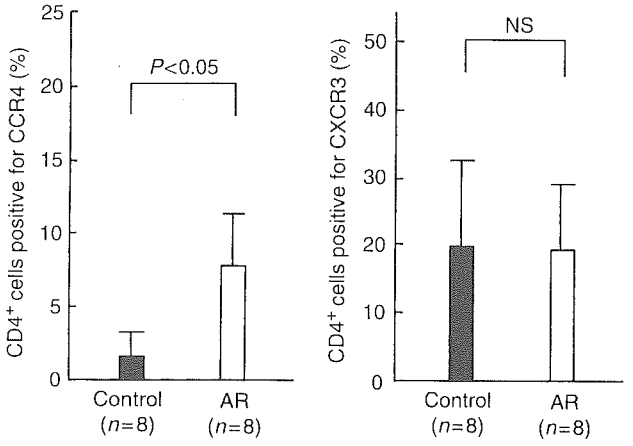

Fig. 1. T cell subpopulations in nasal mucosa of patients with allergic rhinitis and controls. RPE-conjugated anti-CXCR3 and -CCR4 monoclonal antibodies were used as T-helper type 1 (Th1) and Th2 markers, respectively.

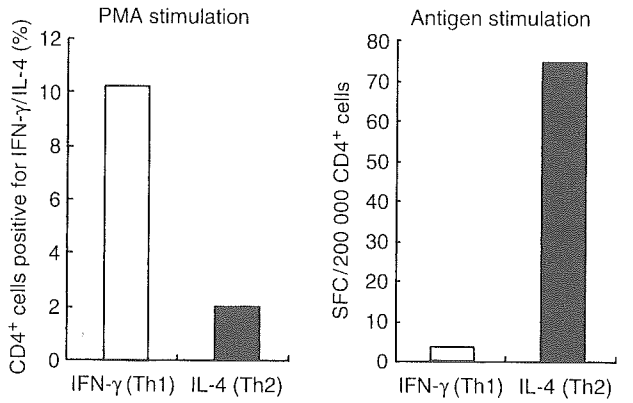

Fig. 3. T cell subpopulations in peripheral blood of a patient with allergic rhinitis. Stimulation by phorbol 12-myristate 13-acetate (PMA) was used to determine the percentage of cells positive for IFN-γ or IL-4. The number of cells secreting IFN-γ and IL-4 were measured by enzyme-linked immunospot. SFC, spot-forming cells.

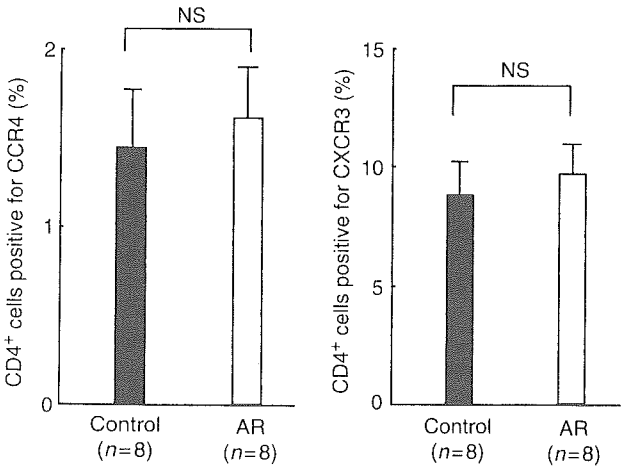

Fig. 2. T cell subpopulations in peripheral blood of patients with allergic rhinitis and controls. RPE-conjugated anti-CXCR3 and -CCR4 monoclonal antibodies were used as T-helper type 1 (Th1) and Th2 markers, respectively.

ingly, specific Th2 cells were observed more frequently than specific Th1, even though non-specific total Th1 cell numbers were higher than those of Th2. Taken together, Th1/Th2 dysregulation is thought to be fundamental to the pathogenesis of AR, although very small numbers of T cells are allergen specific (Fig. 3). Therefore, it seems that levels of antigen specific Th2 cells are increased in AR; antigen-specific Th1/Th2 dysregulation induces antigen-specific IgE synthesis and activation of mast cells and chemokines, which leads to establishment of allergic inflammation.

T-helper type 2 cellular differentiation

Allergens entering the respiratory tract mucosa are captured by antigen-presenting cells (APC), which present them to $CD4^+$ Th cells. APC, which are MHC class II positive, play important roles in antigen-specific Th2 development. Various kinds of cells including monocytes/macrophages, B cells, Langerhans cells and dendritic cells (DC) have APC ability. B cells are one of the major APC in AR [8, 9], but significant numbers of Langerhans cells and DC exist in nasal mucosa and increase dramatically after allergen exposure [10, 11]. These APC including DC are thought to migrate to regional secondary lymphoid organs where they present processed antigen to Th cells in a groove of the MHC class II molecule. It is generally believed that the most important conditions of antigenic presentation are the presence of IL-4 and absence of IL-12 at the presentation site and implication of certain co-receptors determining T cell differentiation to Th2 cells. The exact source of this required IL-4 is not known, but mast cells, basophils, γδ T cells and natural killer T (NKT) cells are thought to be contributors to the Th2 response. NKT cells constitute a novel lymphoid lineage distinct from T, B and NK cells in the immune system. NKT cells are characterized by coexpression of NK1.1 NK receptor and a single invariant antigen receptor encoded by V14-J28 segments in mouse and invariant V24-J15 T cell receptor (TCR) in human. When activated, NKT cells rapidly produce large quantities of IL-4 and IFN-γ, which seem to influence subsequent adaptive immune responses and polarization of conventional-TCR αβ T cells. Recently, it has been shown that activated NKT cells in NKT-deficient mice exert a potent inhibitory effect on Th2 cell differentiation and subsequent IgE production by generating a large amount of IFN-γ. NKT cells play a crucial role in regulating the development of Th2-biased respiratory immunity against nominal exogenous antigens [12, 13].

Co-stimulatory factors can also influence Th1/Th2 differentiation, mainly by modulating contact-dependent factors, among which the extent of TCR ligation and signals delivered by OX4OL–OX40 and B7–CD28 interactions appear most important. Okano et al. have shown that B7 molecules on peripheral circulating T and B cells are up-regulated in AR; moreover, in nasal specimens, B7 molecules and their counter receptor CD28/CD152 are increased in AR patients following nasal provocation with allergen [14, 15].

Recent studies in mice have suggested the presence of several types of regulatory T cells including transforming growth factor β-inducing Th3 cells and IL-10-producing

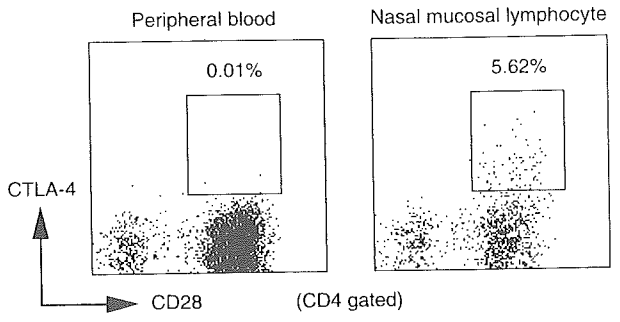

Fig. 4. Analysis of T cells in peripheral blood and mucosal lymphocyte from a patient with allergic rhinitis. CD4-gated cells are shown. The percentage of cells double positive for CD28 and CTLA-4 were 0.01% in peripheral blood and 5.62% in nasal mucosal lymphocyte, respectively.

T regulatory cells. Sakaguchi et al. [16] identified $CD4^+CD25^+$ cells that prevent $CD4^+$ T cell-mediated organ-specific autoimmune diseases. This suppression might be mediated by cell–cell interactions through CTLA-4. Our phenotype analyses of nasal T cells have shown that $CD4^+CD25^+$ $CTLA\text{-}4^+$ cells are present in mucosa of patients with perennial rhinitis but not in mucosa and peripheral circulation of non-AR patients (Fig. 4). Although, the role of $CD4^+CD25^+$ cells in allergic disease remains unclear, Iwamoto's group has reported that these T cells modulate Th1/Th2 cell balance towards Th2 cells and thus up-regulate Th2 cell-mediated allergic inflammation in the airway [17].

New treatments to improve T-helper type 2 dysregulation

To improve Th2 dysregulation, administration of Th1 cytokines or of anti-Th2 antibodies was considered to be a possible useful approach; initial experiments in animals produced some favourable results. However, clinical trials thus far have been disappointing. Administration of anti-IL-5 antibodies to patients with mild asthma significantly reduced eosinophilic infiltration in sputum but did not improve bronchial hyper-reactivity. IL-12 administration to patients with asthma also resulted in limited improvement of symptoms, and intramuscular administration of IFN-γ to patients with pollinosis did not improve symptoms or reduce IgE levels in the serum. These poor results may be because of difficulties with administering sufficient doses of Th1 cytokines and anti-Th2 antibodies to patients. Furthermore, it seems that the differences between the pathogenetic mechanisms of experimentally prepared animal models of hypersensitivity and of human allergy are too great for the successful clinical application of these treatments.

The innate immune system is the host's first line of defence and the early or initial responder to various environmental insults as diverse as Gram-positive and Gram-negative bacteria, viruses and fungi. Immune competent cells such as macrophages, DC, neutrophils and endothelial cells recognize pathogen-associated molecular patterns on the surfaces of pathogens. Toll-like receptors (TLR) are a family of pattern-recognition receptors. A wide diversity of microbial components trigger DC to produce IL-12 via TLR recognition. TLR react to components of bacterial cell wall lipopolysaccharide/lipotechoic acid and bacterial DNA such as CpG motif. IL-12 is a key cytokine influencing Th1/Th2 balance. Environmental factors may influence differentiation of allergen-specific T cells into Th2 phenotype. Shirakawa et al. [18] have demonstrated an inverse association between tuberculin responses and atopic disorders. Indeed, neonatal bacille Calmette Guérin vaccination has been shown to be associated with a lower prevalence of current asthma in a cohort study [19], and Shimada et al. [20] have demonstrated that enteric lactic acid bacteria can influence AR. In light of these results, immunotherapy with specific antigen administration and vaccine therapy using bacterial products have been attempted as ways to improve antigen-specific Th1/Th2 dysregulation [21]. Phase I/IIa studies of T cell peptide therapy against Japanese cedar pollinosis are now underway in Japan. The clinical CpG vaccine study for ragweed pollinosis in USA has shown encouraging results, and similar trials are being prepared to be conducted in Japanese patients.

Conclusions

The ratio of $Th1/CD4^+$ T cells is significantly higher in nasal mucosa of patients with AR than those without. Total numbers of Th1 and Th2 cells in peripheral blood are normal in AR patients. However, levels of antigen-specific Th2 cells are dramatically increased in allergic patients, with concomitant production of antigen-specific IgE leading to the establishment of allergic inflammation. To improve Th1/Th2 dysregulation, various new treatment approaches are under investigation, although on the whole, their effectiveness has yet to be seen.

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Bcl6 regulates Th2 type cytokine productions by mast cells activated by FcεRI/IgE cross-linking

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Abstract

Bcl6-deficient ($Bcl6^{-/-}$) mice displayed Th2 type inflammation, which caused by abnormality of non-lymphoid cells. However, initiators for the Th2 type inflammation were not clear. In order to elucidate the initiators, we investigated property and function of mast cells derived from $Bcl6^{-/-}$ mice. Mast cells were developed from bone marrow cells cultured with IL-3 (BMMCs). Although the development of BMMCs from $Bcl6^{-/-}$ mice was similar to that from wild-type mice, proliferation of $Bcl6^{-/-}$ BMMCs stimulated with IL-3 was slightly lower than that of wild-type BMMCs. When these BMMCs were stimulated by FcεRI/IgE cross-linking, $Bcl6^{-/-}$ BMMCs produced Th2 cytokines more than wild-type BMMCs did. Thus, $Bcl6^{-/-}$ mast cells are one of the initiators for Th2 type inflammation in $Bcl6^{-/-}$ mice, and Bcl6 may be a molecular target for Th2 type allergic diseases.

Keywords: Bcl6; Mast cells; Th2 type cytokines; FcεRI

1. Introduction

The human proto-oncogene *Bcl6* has been identified from chromosomal breakpoints involving 3q27 in diffuse large B-cell lymphomas (Kerchaert et al., 1993; Ye et al., 1993; Miki et al., 1994). The *Bcl6* gene encodes a 92- to 98 kDa nuclear phosphoprotein that contains the BTB/POZ domain in the NH_2-terminal region and *Krüppel*-type zinc finger motifs in the COOH-terminal region. Since the NH_2-terminal half of Bcl6 can bind to silencing mediator of retinoid and thyroid receptor protein (SMRT) and recruit the SMRT/histone deacetylase complex to silencer regions of target genes to repress expression of these genes, *Bcl6* can function as a sequence specific transcriptional repressor. To observe physiological functions of *Bcl6*, this gene was disrupted in the mouse germ line. Bcl6-deficient ($Bcl6^{-/-}$) mice showed growth retardation and abnormal immune responses. They completely lack germinal center formation (Dent et al., 1997; Fukuda et al., 1997; Ye et al., 1997) and affinity maturation of antibodies specific for immunized antigens (Toyama et al., 2002).

$Bcl6^{-/-}$ mice also displayed inflammatory responses in multiple organs, especially in heart and lungs, characterized by infiltration of eosinophils and IgE bearing B lymphocytes (Th2 type inflammation), and frequently die at an early adult age (Dent et al., 1997; Ye et al., 1997; Yoshida et al., 1999). Immunization of $Bcl6^{-/-}$ mice with a protein antigen in adjuvant accelerated induction of the Th2 type inflammation (Ye et al., 1997). These data suggested that the Th2 type inflammation was originated by antigen-activated cells, especially

Abbreviations: BMMCs, bone marrow derived mast cells; $Bcl6^{-/-}$, Bcl6-deficient; SMRT, silencing mediator of retinoid and thyroid receptor protein; WT, wild-type; DNP, dinitrophenyl; HAS, human serum albumin; PI, propidium iodide

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T cells. Indeed, we have recently reported that the *IL-5* gene is one of the molecular targets of Bcl6 in T cells (Arima et al., 2002). Furthermore, recent analysis of the mechanism for the Th2 type inflammation using $Bcl6^{-/-}$ embryonic stem cell-derived chimeric mice indicated that the functional dominance of Th2 cells in $Bcl6^{-/-}$ mice is due to non-lymphoid cells including macrophages, dendritic cells, and mast cells (Toney et al., 2000). However, initiators for the Th2 type inflammation were not clear.

Mast cells are distributed throughout the vascularized tissues and play a key role in allergic reactions by releasing proinflammatory mediators such as histamine, leukotrienes and prostaglandin by antigen stimulation though a FcεRI/IgE complex. Mast cells also play a central role in innate immunity (Supajatura et al., 2002) or induce autoantibody-mediated vasculitis (Malaviya et al., 1996) by TNFα production. Furthermore, mast cells effect on immune competent cells, such as survival of eosinophils, maturation of dendritic cells, and activation of B and T cells by production of cytokines (Levi-Schaffer et al., 1998; Skokos et al., 2001; Skokos et al., 2003). Mast cells stimulated by FcεRI/IgE cross-linking produce the large amount of IL-4, which is essential for the induction of Th2 development (Schmitz et al., 1994; Fallon et al., 2002), and chemokines such as MCP-1, MIP1α, RANTES and Eotaxin, which work on Th2 development and eosinophil recruitment (Nakajima et al., 2002). These results suggest that mast cells seem to be an initiator of the Th2 type inflammation in $Bcl6^{-/-}$ mice. Therefore, we investigated property and function of $Bcl6^{-/-}$ mast cells derived from bone marrow cells cultured with IL-3 (BMMCs). When BMMCs were stimulated by FcεRI/IgE cross-linking, Th2 type cytokine productions by the $Bcl6^{-/-}$ BMMCs were larger than those by the wild-type (WT) BMMCs. We discuss a role for Bcl6 in function of mast cells.

2. Materials and methods

2.1. Mice

Bcl6-deficient ($Bcl6^{-/-}$) (Yoshida et al., 1999), (C57BL/6 × DBA2) F1(Japan SLC), C57BL/6 mice (Japan SLC) were housed in microisolate cages under pathogen free conditions. All experiments were performed according to the guidelines of Graduate School of Medicine, Chiba University (Chiba, Japan).

2.2. Culture of BMMCs

Femoral bone marrow cells of 8–12-week-old $Bcl6^{-/-}$ and WT mice were isolated and cultured in tissue culture plates as described previously (Suzuki et al., 2000). These bone marrow cells were cultured in RPMI 1640 medium containing 10% FCS and 5–10% of murine IL-3 conditioned medium (x63-IL3; kindly provided from Dr. H. Karasuyama, Tokyo Medical and Dental University) at 37 °C. Non-adherent cells in the culture were harvested every week and continuously cultured in the new plates with fresh medium.

2.3. Flow cytometry

For detection of c-Kit and FcεRI on BMMCs, BMMCs were first incubated with un-conjugated anti-CD32/16 monoclonal antibody (2.4G2; BD PharMingen, San Diego, CA) at 4 °C for 10 min, then with IgE anti-dinitrophenyl (DNP) monoclonal antibody (SPE-7; Sigma, St Louis, MO) for 50 min, followed by the incubation with biotin conjugated anti-mouse IgE antibody (BD PharMingen) for 10 min. These cells were further incubated with streptavidin-conjugated allophycocyanin (BD PharMingen) and phycoerythrin conjugated anti-c-Kit monoclonal antibody (BD PharMingen) for 10 min. Flow cytometric analysis was performed using a FACSCalibur (Becton Dickinson, San Jose, CA).

2.4. Cell survival assay

BMMCs were washed 3 times with PBS and cultured in a 96-well-plate (1.5×10^6 cells/ml) with RPMI 1640 medium without IL-3 at 37 °C for 8 days. Viability of these cultured cells was assessed by trypan blue exclusion method, and the number of viable cells was counted on day 0, 1, 3, 5 and 8 after stimulation. For analysis of apoptosis, BMMCs were incubated with FITC-labeled annexin V and propidium iodide (PI) (Bender Medsystems, Vienna, Austria) for 15 min according to the manufacturer's instructions. Analysis of apoptosis was performed using a FACSCalibur.

2.5. Proliferation assay

BMMCs were washed 3 times with PBS and cultured in a 96-well-plate (1.0×10^6 cells/ml) with RPMI 1640 medium containing 0–10% of IL-3 at 37 °C for 36 h. These cells were incubated with 1 μCi [^{3}H]thymidine (Amersham International, Aylesbury, UK) during the last 12 h. These cells were harvested onto glass fiber filters, and [^{3}H]thymidine was counted with a liquid scintillation counter.

2.6. Stimulation of BMMCs by FcεRI/IgE cross-linking

BMMCs (1×10^6 cells/ml) were incubated with IgE anti-DNP antibody (1 μg/ml) overnight at 37 °C and washed twice with RPMI medium (Malaviya and Uckun, 2002). These cells were then challenged with various concentrations of DNP–human serum albumin (DNP–HSA; Sigma) for 30 min.

2.7. β-hexosaminidase release assay

BMMCs were stimulated by FcεRI/IgE cross-linking in Tyrode buffer (130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 10 mM HEPES, and 0.1% BSA, pH 7.4). The amount of β-hexosaminidase in culture supernatants and cell lysates of the activated BMMCs

was measured by hydrolysis of *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside (Sigma) in 0.1 M sodium citrate buffer (pH 4.5) for 2 h at 37 °C. The reaction was stopped by addition of 0.2 M glycine and the optical density at 405 nm of the reaction mixture was measured by a densitometry. The percentage of specific β-hexosaminidase release was calculated as follows: the percentage released = 100 × (activity in supernatants/activity in supernatants and lysates) as previously described (Watanabe et al., 1999).

2.8. RT-PCR for cytokine mRNAs

Total RNA was isolated from BMMCs using TRIzol Reagent (GIBCO-BRL, Burlington, Canada) according to the manufacturer's instructions. The cDNA was synthesized from total mRNA (1 μg) with oligo(dT)$_{12-18}$ as a primer and Superscript II RNase H reverse transcriptase (Life Technologies, Rockville, MD). Levels of cytokine mRNA were assessed by semi quantitative RT-PCR. The 20 cycles of PCR for β-actin and 30 cycles of PCR for cytokines were carried out using the following conditions: denaturation at 94 °C for 30 s, annealing at 55 °C for 40 s and polymerization at 72 °C for 60 s. PCR primers for the cDNA amplification were as follows: *IL-4* primers, 5′-ACTGACGGCACAGAGCTATTGATG-3′ and 5′-GGACTCATTCATGGTGCAGCTTATC-3′; *IL-5* primers, 5′-AGGATGCTTCTGCACTTGAGTGTTC-3′ and 5′-CCCTTGCATTTGCACAGTTTTG-3′; *IL-6* primers, 5′-GTTCTCTGGGAAATCGTGGA-3′ and 5′-TGTACTCCAGGTAGCTATGG-3′; *IL-13* primers, 5′-CAGTCCTGGCTCTTGCTTGC-3′ and 5′-AAGTGGGCTACTTCGATTTTGG-3′; *TNFα* primers, 5′-TCTCATCAGTTCTATGGCCC-3′ and 5′-GGGAGTAGACAAGGTACAAC-3′; *β-actin* primers, 5′-GTTTGAGACCTTCAACACC-3′ and 5′-GTGGCCATCTCCTGCTCGAAGTC-3′.

2.9. ELISA

Concentration of IL-6 (OptEIA, San Diego, CA) and IL-13 (R&D Systems, Minneapolis, MN) in culture supernatants was measured by ELISA according to the manufacturer's instructions.

2.10. Statistical analysis

Statistical analysis was made using unpaired *t*-test. *P*-values of <0.05 were considered to be significant.

3. Results

3.1. Development of BMMCs from Bcl6$^{-/-}$ bone marrow cells stimulated with IL-3

Bcl6$^{-/-}$ mouse peritoneal mast cells were stained with antibodies to FcεRI and to c-Kit, and analyzed on a FACS.

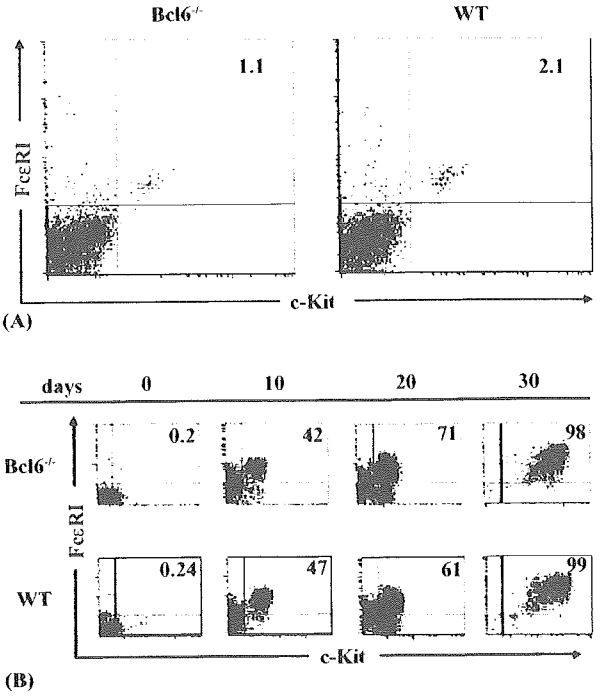


Fig. 1. Development of Bcl6$^{-/-}$ BMMCs. (A) Mast cells in peritoneal cavity of Bcl6$^{-/-}$ and WT mice were assessed by FACS analysis. The numbers in the corners indicate the percentages of mast cells in total peritoneal cells. (B) Bone marrow cells of Bcl6$^{-/-}$ and WT mice were cultured with IL-3. Development of mast cells was assessed by FACS analysis. The numbers in the corners indicate the percentages of mast cells in cultured cells.

Although the percentage of Bcl6$^{-/-}$ peritoneal mast cells was less than that of WT ones because of the larger cell infiltrations in the peritoneal cavity of Bcl6$^{-/-}$ mice, mast cells with normal surface phenotypes were observed in Bcl6$^{-/-}$ mice as well as WT littermates (Fig. 1A). In order to examine development of mast cells from bone marrow cells, Bcl6$^{-/-}$ bone marrow cells were cultured with IL-3 for 30 days. Mast cells developed in the culture were stained with anti-c-Kit and anti-FcεRI antibodies and analyzed on a FACS every 10 days. Mast cells, with more than 98% of purity, were developed in the Bcl6$^{-/-}$ bone marrow cell cultures as well as in the WT ones within 30 days (Fig. 1B). The level of FcεRI and c-Kit on Bcl6$^{-/-}$ BMMCs was similar to that on WT BMMCs, and percentages of Bcl6$^{-/-}$ BMMCs (c-Kit^{+}, FcεRI^{+}) in the cultures from day 10 to day 30 were similar to those of WT BMMCs. Thus, the deficiency of Bcl6 in bone marrow cells may not affect the development of mast cells.

3.2. Functional property of Bcl6$^{-/-}$ BMMCs

Since IL-3 is known to be an important mast cell growth factor, proliferation of Bcl6$^{-/-}$ BMMCs to IL-3 stimulation was examined by thymidine uptake. Proliferation of Bcl6$^{-/-}$ BMMCs stimulated with IL-3 was slightly lower than that of WT BMMCs (Fig. 2).

Apoptosis is induced in BMMCs by IL-3 starvation (Suzuki et al., 2000), and almost all of Bcl6$^{-/-}$ and WT BMMCs died within 8 days after IL-3 starvation (Fig. 3A).

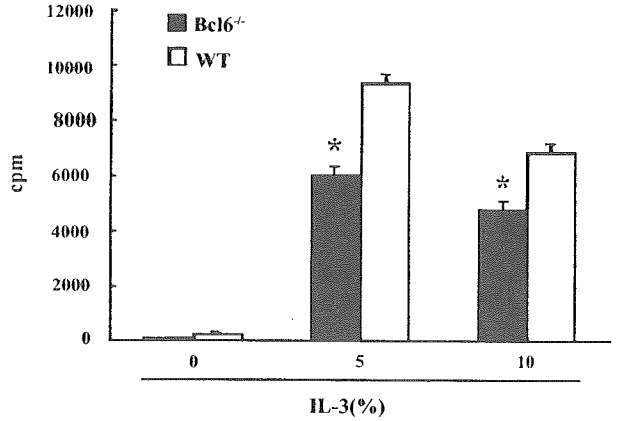


Fig. 2. Proliferation of Bcl6$^{-/-}$ BMMCs. Bcl6$^{-/-}$ (closed bar) and WT (open bar) BMMCs were cultured in the presence of various doses of IL-3 for 36 h. Data represents the mean ± S.D. of triplicate cultures. $^{*}P < 0.0001$. Results are representative of three independent experiments.

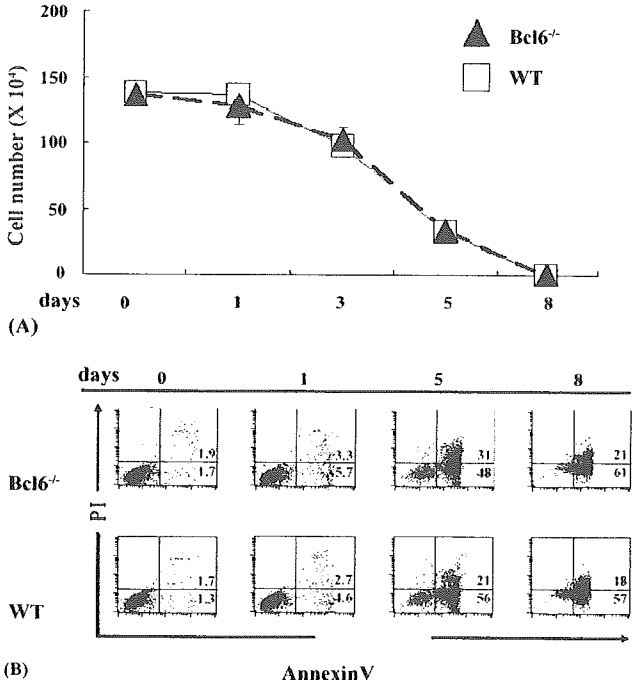


Fig. 3. Apoptosis of Bcl6$^{-/-}$ BMMCs by IL-3 starvation. (A) Live cell numbers of Bcl6$^{-/-}$ (closed triangle) and WT (open square) BMMCs were measured by die exclusion method after IL-3 starvation. (B) Apoptosis of Bcl6$^{-/-}$ and WT BMMCs after IL-3 starvation was detected by staining with annexin V and PI. The numbers in the corners indicate the percentages of cells in each quadrant. Results are representative of three independent experiments.

Thus, apoptosis of Bcl6$^{-/-}$ BMMCs after IL-3 starvation was examined by annexin V and PI staining. Percentages of apoptotic cells in Bcl6$^{-/-}$ BMMCs were similar to those in WT BMMCs until day 8 after starvation (Fig. 3B).

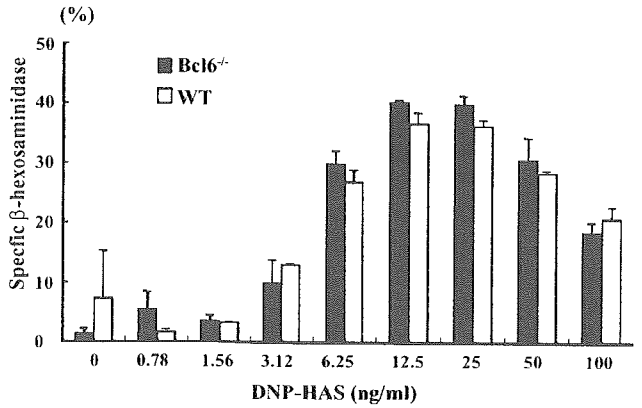


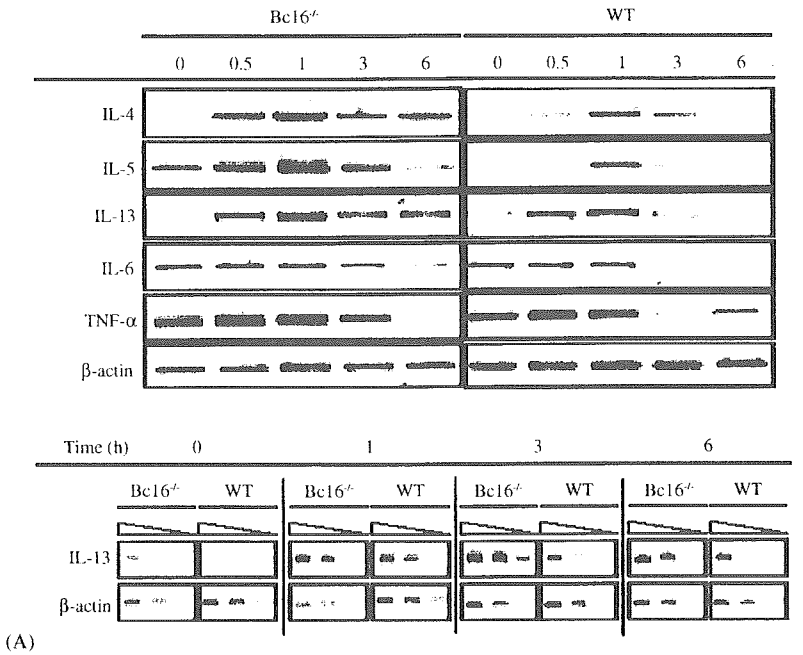
Fig. 4. Degranulation of Bcl6$^{-/-}$ BMMCs. Degranulation of BMMCs stimulated by FcεRI/IgE cross-linking was assessed by the β-hexosaminidase release assay. After Bcl6$^{-/-}$ (closed bar) and WT (open bar) BMMCs were stimulated by FcεRI/IgE cross-linking with various doses of DNP–HAS, the release of β-hexosaminidase in the culture supernatants was measured. Data represents the mean ± S.D. of triplicate cultures. Results are representative of three independent experiments.

FcεRI/IgE cross-linking is one of the major stimulations of mast cells, induces degranulation and chemical mediators release from mast cells. Since mouse mast cells release β-hexosaminidase as the result of FcεRI/IgE cross-linking (Watanabe et al., 1999; Suzuki et al., 2000), a release of β-hexosaminidase by Bcl6$^{-/-}$ BMMCs after FcεRI/IgE cross-linking was examined by the β-hexosaminidase release assay. As shown in Fig. 4, Bcl6$^{-/-}$ BMMCs released the similar amount of β-hexosaminidase as WT BMMCs did after FcεRI/IgE cross-linking with any amounts of DNP-HSA examined. Thus, degranulation of mast cells after FcεRI/IgE cross-linking is Bcl6-independent.

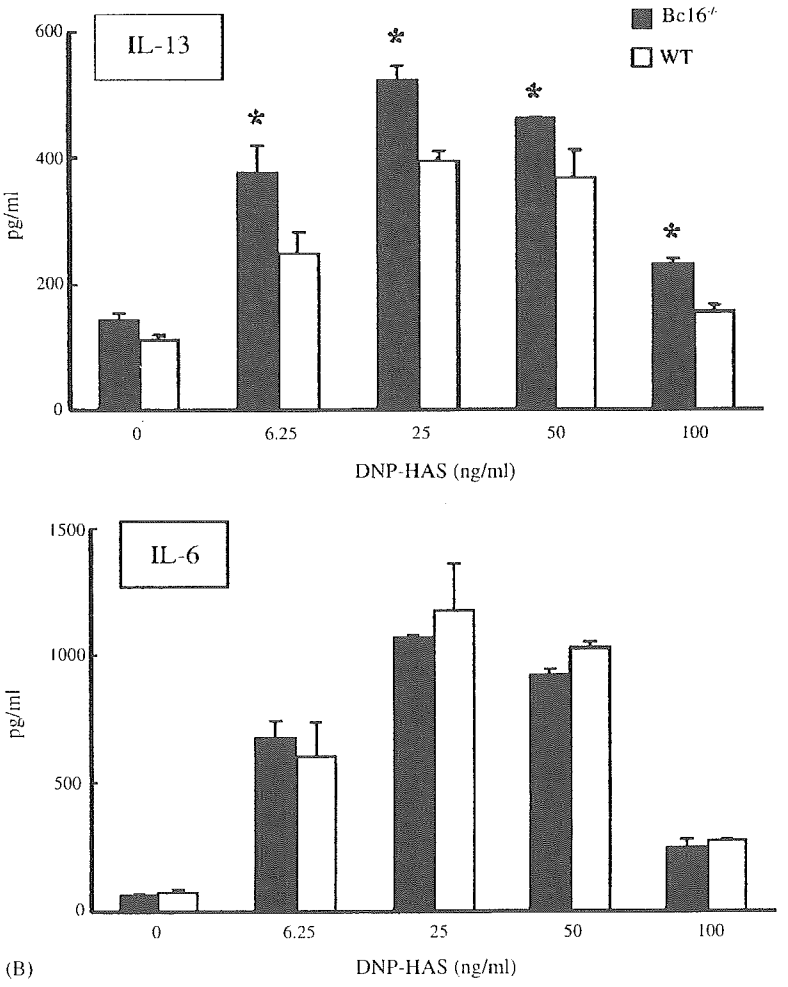
3.3. *Th2 cytokine productions of Bcl6$^{-/-}$ BMMCs stimulated by FcεRI/IgE cross-linking*

Since Th2 cytokine mRNA expression is augmented in Bcl6$^{-/-}$ T cells (Dent et al., 1997), we examined Th2 cytokine mRNA expression in Bcl6$^{-/-}$ BMMCs stimulated by FcεRI/IgE cross-linking by RT-PCR. Expression of *IL-4* and *IL-5* mRNA was clearly augmented in Bcl6$^{-/-}$ BMMCs after stimulation (Fig. 5A). Since expression of *IL-13* mRNA in Bcl6$^{-/-}$ BMMCs seemed to be more than that in WT BMMCs 3 and 6 h after stimulation, the amount of *IL-13* mRNA in those activated BMMCs was carefully examined by semi-quantitative RT-PCR. The amount of *IL-13* mRNA in Bcl6$^{-/-}$ BMMCs was 3- to 10-fold more than that in WT BMMCs 3 and 6 h after stimulation. However, there was no significant difference of *IL-6* and *TNFα* mRNA expression between Bcl6$^{-/-}$ BMMCs and WT BMMCs after stimulation.

Fig. 5. Production of Th2 cytokines by Bcl6$^{-/-}$ BMMCs. BMMCs were stimulated by FcεRI/IgE cross-linking with DNP–HAS (25 ng/ml). (A) Expression of Th2 cytokines in these BMMCs was assessed by RT-PCR (upper panel). The amount of *IL-13* mRNA was measured in cDNA with 3-hold dilutions (lower panel). (B) Production of IL-13 and IL-6 in the culture supernatants 24 h after stimulation was assessed by ELISA. Data represents the mean ± S.D. of triplicate cultures. Results are representative of three independent experiments. $^{*}P < 0.01$.


Bcl6-/-
WT
0 0.5 1 3 6 0 0.5 1 3 6
IL-4
IL-5
IL-13
IL-6
TNF-α
β-actin
Time (h) 0 1 3 6
Bcl6-/- WT Bcl6-/- WT Bcl6-/- WT Bcl6-/- WT
IL-13
β-actin

(A)


IL-13
Bcl6-/-
WT
600
400
200
0
pg/ml
0 6.25 25 50 100
DNP-HAS (ng/ml)
IL-6
1500
1000
500
0
pg/ml
0 6.25 25 50 100
DNP-HAS (ng/ml)

(B)

Next, we examined cytokine productions in culture supernatants of Bcl6$^{-/-}$ BMMCs stimulated by FcεRI/IgE cross-linking by ELISA. The amount of IL-13 in the culture supernatants of Bcl6$^{-/-}$ BMMCs was more than that of WT BMMCs after FcεRI/IgE cross-linking with any amounts of DNP-HSA examined (Fig. 5B). The amount of IL-6 in the culture supernatants of Bcl6$^{-/-}$ BMMCs was similar to that of WT BMMCs after stimulation. However, we could not detect IL-4 and IL-5 in the culture supernatants of Bcl6$^{-/-}$ and WT BMMCs after stimulation (data not shown).

4. Discussion

Bcl6$^{-/-}$ mice showed abnormal Th2 type inflammation (Dent et al., 1997) and the inflammation is initiated by non-lymphoid cells (Toney et al., 2000). In order to elucidate an initiator of the Th2 type inflammation, we examined functional property of mast cells from Bcl6$^{-/-}$ mice as an initiator. There was no significant difference between Bcl6$^{-/-}$ BMMCs and WT BMMCs in expression of surface FcεR1 and c-Kit, in apoptosis induced by deprivation of IL-3, and in degranulation (release of β-hexosaminidase) induced by FcεRI/IgE cross-linking. However, Bcl6$^{-/-}$ BMMCs stimulated by FcεRI/IgE cross-linking expressed more amounts of Th2 cytokine mRNAs such as *IL-4*, *IL-5* and *IL-13* than WT BMMCs did. The increased expression of *IL-13* mRNA in Bcl6$^{-/-}$ BMMCs after stimulation was reflected to the amount of IL-13 protein in culture supernatants of the Bcl6$^{-/-}$ BMMCs. However, we could not detect IL-4 and IL-5 protein in the culture supernatants of Bcl6$^{-/-}$ and WT BMMCs after stimulation. These cytokines may be difficult to detect in supernatants of the BMMC culture by ELISA, because their immunoreactivity may be changed by the heparin produced by BMMCs or these cytokines may be degraded by protease chymase produced by BMMCs as previously reported (Okayama et al., 1995).

Mast cells have been focused on as a source of IL-4 in allergic diseases, and IL-4 is the most potent factor that induces Th2 development (Fallon et al., 2002). However, cell types that produce the initial IL-4 for the induction of Th2 development are controversial. It has been speculated that IL-4 from non-Th cells, such as mast cells and basophiles, may be required for Th2 development. For example, mast cells rapidly release large amounts of Th2 cytokines including IL-4 by FcεRI/IgE cross-linking (Kalesnikoff et al., 2001) or with lipopolysaccharide stimulation (Supajatura et al., 2002), and 90% of IL-4 producing cells in the mucosa of patients with allergic rhinitis are mast cells (Bradding et al., 1992). Since Th2 cytokines, not only IL-4 but also IL-13, are known to be important for Th2 development (McKenzie et al., 1998; Chiaramonte et al., 1999), the mast cells are one of the initiators for Th2 dominancy in Bcl6$^{-/-}$ mice.

Mast cells do not mature in the bone marrow, circulate as committed progenitors and complete their maturation, and are activated in the peripheral tissues. Thus, mast cells are considered to play an important role in peripheral tissues. Mast cells activated by various stimuli including FcεRI/IgE cross-linking and lipopolysaccharide (Kalesnikoff et al., 2001; Nakajima et al., 2002; Supajatura et al., 2002) rapidly release a number of cytokines and chemokines, and recruit and activate other immune competent cells (Levi-Schaffer et al., 1998; Skokos et al., 2001; Skokos et al., 2003). Chemical mediators produced by activated mast cells are also essential for allergic reactions. For example, histamine is an essential mediator for type I allergy and anaphylaxis (Dombrowicz et al., 1993; Naclerio, 1999; White, 1999), leukotrienes are essential for vascular permeability and bronchial reconstruction (Kanaoka et al., 2001; Maekawa et al., 2002), prostaglandin works on microvascular and also bronchial reconstruction (Diaz et al., 2002). Since the number of intraepithelial mast cells increased in Bcl6$^{-/-}$ mice (Toney et al., 2000) and the production of Th2 cytokines was augmented in activated Bcl6$^{-/-}$ mast cells, Bcl6$^{-/-}$ mast cells may also be a major effector for Th2 type inflammation in Bcl6$^{-/-}$ mice.

In summary, Bcl6 regulates Th2 type cytokine productions by mast cells stimulated by FcεRI/IgE cross-linking. Thus, Bcl6$^{-/-}$ mast cells are one of the initiators for Th2 type inflammation in Bcl6$^{-/-}$ mice, and Bcl6 may be a molecular target for Th2 type allergic diseases.

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ORIGINAL ARTICLE

Comparative study of two Japanese rhinoconjunctivitis quality-of-life questionnaires

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Abstract

Conclusion. Two questionnaires were used to assess quality of life (QOL) in allergic rhinitis: the Japanese translation of the Rhino-conjunctivitis Quality of Life Questionnaire (RQLQJ) and an original Japanese QOL questionnaire (JRQLQ). Either questionnaire may be used to assess QOL depending on differences in target domains. *Objectives.* Although pollinosis is a common disease which has a major impact on patient QOL, no internationally standardized questionnaire has been available in Japan until now. The aim of this study was to compare two currently available QOL questionnaires for allergic rhinitis in Japan—the RQLQJ and JRQLQ—in terms of their appropriateness for clinical use and their psychometric properties. *Material and methods.* A multicenter, inter-group, cross-sectional study was conducted in 187 adult symptomatic patients with Japanese cedar pollinosis in 2003. Patient scores on the two questionnaires were compared in terms of both overall and comparable domains. We also examined the acceptability, construct and reliability of both questionnaires. *Results.* The questionnaires were highly correlated in terms of both overall and comparable domain scores. In addition, both questionnaires had equal and satisfactory psychometric validity, demonstrating that they are both useful tools for assessing QOL in rhinitis. However, when compared with each other, the JRQLQ focuses mainly on activities of daily life and is simpler, while the RQLQJ focuses mainly on rhinitis-related health and is more responsive.

Keywords: *Quality of life, questionnaires, rhinoconjunctivitis*

Introduction

The evaluation of health-related quality of life (QOL) has been recognized as essential to a comprehensive and holistic evaluation of diseases and treatments. Although not life-threatening, the negative impact of allergic rhinoconjunctivitis (AR) on patients' QOL is generally acknowledged.

QOL is usually assessed using generic or disease-specific self-administered questionnaires. Although various AR-specific questionnaires have been available internationally [1], until recently there have been no useful questionnaires of high quality in Japan. Of the available AR-specific questionnaires, the Rhino-conjunctivitis Quality of Life Questionnaire (RQLQ) devised by Juniper [2] is most commonly used internationally. However, the RQLQ, which was originally developed in English, must be culturally and linguistically adapted for Japanese patients following an internationally recognized methodology. Currently, the RQLQ has been translated and validated for use in Japan (RQLQJ) [3,4]. In addition, we have also developed the JRQLQ, a de novo Japanese QOL questionnaire for AR. The JRQLQ has been shown to have satisfactory acceptability, construct, concurrent and clinical validities and reliability [5]. Moreover, both the RQLQJ and JRQLQ seem to be equally valuable for assessing QOL in rhinitis patients. However, they

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differ in terms of their constructions, scale assessments and principal domains. The purpose of this study was to elucidate the relationships between the two questionnaires.

Material and methods

Subjects and administration of questionnaires

A multicenter, inter-group, comparative, cross-sectional study was conducted in 187 adult symptomatic patients with Japanese cedar pollinosis (JCP) during the 2003 pollen season (February to April) at 10 institutions in Japan. We tested two self-administered questionnaires (the RQLQJ and JRQLQ) simultaneously in the same patients.

Prior to the study, written informed consent for QOL evaluation was obtained from the patients and they were assured that their privacy would be protected. The decision regarding which questionnaire was tested first was randomly determined based on clinical record numbers. Patients were then asked to complete the questionnaires sequentially and without any assistance from physicians or nurses, with the exception of noting missing data, when necessary. The local Institutional Review Boards at Nippon Medical School approved the study from the ethical point of view.

Scoring

The questionnaire domains were scored according to the developer's recommendations. The domain scores were calculated as the mean of the total item scores. Each item score ranged from zero to six for the RQLQJ and from zero to four for the JRQLQ, with lower scores reflecting better health-related QOL. Overall scores were found by summing the means of all the items. A total symptom score was calculated for each questionnaire as the total of three nasal plus two eye symptom scores in the RQLQJ and as the total of four nasal plus two eye symptom scores in the JRQLQ. The items and domains for both questionnaires are presented in Table II.

Data analysis

The correlations of the mean scores between each of the domains in both questionnaires were investigated and compared with each other using Spearman's test. However, because the questionnaires differed in terms of their content, the correlations of the mean scores were also examined between matched comparable domains and items in both questionnaires.

The following JRQLQ and RQLQJ domains were matched and compared: nasal/ocular symptoms in the JRQLQ and nose and eye symptoms in the RQLQJ; usual daily activity in the JRQLQ and activity limitation in the RQLQJ; sleep problems; emotional function.

It is important to note that the activity limitation domain in the RQLQJ consists of 3 items selected by individual patients from 30 example items. These example items are included in similar domains in the JRQLQ, such as usual daily activity, outdoor activity and social functioning.

Psychometric evaluation

Validation studies of both questionnaires were conducted previously [3–5]. However, there has never been a direct comparison of the psychometric properties of the two questionnaires. Therefore, the validity of both questionnaires was confirmed and compared in this study by examining the following psychometric properties: acceptability; clinical validity; factor analysis; item–domain correlations (convergent and discriminate validity); and internal consistency reliability (reliability validity) [6–8]. Acceptability was defined as the percentage of missing data for individual items. To determine clinical validity, the correlation between nose and eye symptom levels and individual QOL domain scores was assessed using Spearman's test. The symptom levels were scored as mentioned above. Correlation coefficients were considered to be satisfactory when they were ≥ 0.4. To determine construct validity, factor and multitrait analyses were used. In multitrait analysis, convergent validity was tested in relation to discriminate validity. An item is expected to have stronger relationships with its own factor and weaker relationships with others. Correlations between each item and its own domain should be ≥ 0.4 [8]. Moreover, each item should have a higher correlation with its own domain than with any other domain. For factor analysis, factor loading with Varimax rotation, the communalities and the number of factors required have to be estimated from the data, usually from the sample correlation matrix. To test reliability, the internal consistency reliability was assessed using Cronbach's alpha [9]. Floor and ceiling effects (the percentages of subjects with the lowest and highest possible scores, respectively) were also calculated [9].

$p < 0.05$ was considered to significant. Other statistics are described in the relevant sections below. The software used were Excel STAT 2002 and Stat View version 4.

Table I. Patient demographics.

Sex (M:F)	49.7%:50.3%
Age (years); mean ± SD	40.6 ± 12.7
Type of AR (seasonal:seasonal + perennial)	86.6%:13.4%
Symptom score[a] (≥12: <12)	64.2%:35.8%

[a]Total nose/eye symptom score on the JRQLQ.

Results

The patient demographics and their total symptom scores (the ratio of scores of ≥12 to <12) are presented in Table I.

The mean domain score distributions for the JQRQL and RQLQJ were roughly equivalent. The numbers of items in each domain and the mean total domain scores are presented in Tables II and III.

The percentage of missing data for individual items was negligible for both the JRQLQ and RQLQJ (<1%), with the exception of the RQLQJ activity limitation domain (7.6% of total subjects), indicating good acceptability.

The correlations between domain scores for the two questionnaires were satisfactory ($r > 0.4$), with higher correlations being found for similar domains, as follows: activity limitation and usual daily activity; outdoor activity domains; sleep problem domains; non-nose/eye symptoms and general health; emotional domains (Table IV). Better correlations were obtained when the domains were adjusted for matching, as described above (Table V). Almost all correlation coefficients between domains of the two questionnaires were >0.6. These results indicate

Table II. Domain components and items in the JRQLQ and the RQLQJ.

JRQLQ	RQLQJ
1. Usual daily activities	1. Activities limitation
Reduced productivity at work/school/home	These items selected from 30 suggested, including usual daily activity, outdoor activity and social functioning
Poor mental concentration	2. Practical problems
Reduced thinking power	Inconvenience of carrying tissues paper or handkerchief
Impaired reading of book/, newspapers	Need to rub nose/eyes
Poor memory	Need to blow nose repeatedly
2. Outdoor activities	3. Sleep problem
Limitation of outdoor life	Difficulty getting to sleep
Limitation of going out	Waking up during night
3. Social functioning	Lack of a good night's sleep
Reluctance of visiting friends/ relatives	4. Non-nose/eye symptoms
Reduced contact with friends or others by telephone or in person	Tiredness
Being uneasy with people around you	Fatigue
4. Sleep problems	Thirst
Impaired sleeping	Reduced productivity
5. General health problems	Poor concentration
Tiredness	Worn out
Fatigue	Headache
6. Emotional function	5. Emotional function
Frustrated	Frustrated
Irritable	Irritable
Depressed	Impatient or restless
Unhappy	Embarrassed
7. Nasal-ocular symptoms[a]	6. Nose symptoms
Stuffy nose	Stuffy nose
Runny nose	Runny nose
Sneezing	Sneezing
Itchy eyes	Postnasal drip
Watery eyes	7. Eye symptoms
	Itchy eyes
	Watery eyes
	Sore eyes
	Swollen eyes

[a]Not included in the overall score in the JRQLQ.