Allergology International. 2008;57:147-155
DOI: 10.2332/allergolint.O-07-510

ORIGINAL ARTICLE

Enhanced Production of IgE Anti-Japanese Cedar Pollen Specific Antibodies by Peripheral Blood B Cells from Patients with Japanese Cedar Pollinosis

Nobuharu Ohshima[1] and Shunsei Hirohata[2]

ABSTRACT

Background: IgE antibodies against Japanese cedar pollen (JCP) play an important role for the pathogenesis of the cedar pollinosis, but the mechanism of their production has been unclear. We explored the capacity of peripheral blood B cells from pollinosis patients to produce anti-JCP specific IgE.

Methods: Peripheral blood B cells from 16 pollinosis patients and 9 normal subjects were cultured with mitomycin-C treated T cells with immobilized anti-CD3 for 10 days.

Results: B cells from pollinosis patients produced higher amounts of anti-JCP specific IgE than those from normal subjects upon stimulation with immobilized anti-CD3 activated autologous T cell, whereas the production of anti-JCP specific IgM were comparable between normal subjects and patients. Exogenous IL-4/IL-5 or anti-CD3 stimulated patients' T cells could not induce the production of anti-JCP specific IgE from normal B cells.

Conclusions: These results indicate that B cells from normal individuals contain comparable numbers of precursors that are committed to produce anti-JCP specific IgM to patients' B cells. Moreover, the data confirm that the class switching of IgM to IgE within anti-JCP specific B cells contributes to development of Japanese cedar pollinosis.

KEY WORDS

B cells, class switching, ELISA, immobilized anti-CD3, Japanese cedar pollen

INTRODUCTION

The number of patients with Japanese cedar pollinosis is still increasing every year since the first report in 1964.[1] Thus, Japanese cedar pollinosis has become one of the serious social problems in recent years, since as many as 20% of the Japanese population are suffering from this disease.

A number of studies have shown that IgE antibodies against Japanese cedar pollen (JCP) play an important role in the pathogenesis of Japanese cedar pollinosis.[2-4] The mechanism of its production remains still unclear, although it is suggested that the production of anti-JCP specific IgE might be induced through class-switching from IgM anti-JCP producing B cells to IgE anti-JCP producing B cells by stimulation with IL-4, IL-13 and CD40L.[5,6] Comparison of the repertoire of peripheral blood B cells against JCP between normal subjects and pollinosis patients is very important in order to elucidate the mechanism involved in production of anti-JCP specific IgE. Shinmoto *et al*. recently reported that most anti-JCP-secreting cells belong to the IgM-producing B cells in normal subjects and even in pollinosis patients, using

[1]Department of Respiratory Medicine, National Hospital Organization, Tokyo National Hospital, Tokyo and [2]The Department of Rheumatology and Infectious Diseases, Kitasato University School of Medicine, Kanagawa, Japan.
Correspondence: Shunsei Hirohata, MD, Department of Rheumatology and Infectious Disease, Kitasato University School of Medicine, 1–15–1 Kitasato, Sagamihara, Kanagawa 228–8555, Japan.
Email: shunsei_tenpoint@yahoo.co.jp
Received 24 July 2007. Accepted for publication 16 October 2007.

the system of transformation of B cells by EB virus.[7] However, in the system of EB virus transformation, only 0.027–0.34% of peripheral blood B cells have been shown to be activated.[8] Thus, it is likely that the clone size of IgE anti-JCP producing B cells might be too small to be activated by EB virus even in pollinosis patients. Therefore, it is still unclear whether there might be class-switched B cells that can produce anti-JCP specific IgE in the peripheral blood of patients with Japanese cedar pollinosis.

We have previously developed a system with immobilized anti-CD3 monoclonal antibodies (mAb) that can provide powerful stimulation of peripheral blood B cells.[9] Thus, in this system as many as 5% of peripheral blood B cells can be stimulated to differentiate into Ig producing B cells.[9] This system therefore might allow us to examine the characteristics of anti-JCP-producing cells in peripheral B cells from normal subjects and pollinosis patients. The current studies were designed to examine whether peripheral blood B cells from Japanese pollinosis patients have higher capacity to produce anti-JCP specific IgE than those from normal subjects.

METHODS

DETERMINATION OF IGE JCP-SPECIFIC ANTIBODIES (RAST)

Specific IgE antibodies to JCP in sera were determined by quantitative CAP-RAST-FEIA (Pharmacia, Uppsala, Sweden) measurements.

PATIENTS AND SAMPLES

Peripheral blood was obtained from 16 pollinosis patients and 9 normal subjects, who gave informed consent. The 16 patients consisted of 12 males aged 27–57 (mean 34 years) and 4 females aged 26–36 (mean 31 years), and the 9 normal subjects consisted of 6 males aged 27–48 (mean 34 years) and 3 females aged 24–26 (mean 25 years). All the 16 pollinosis patients presented sneezing, nasal congestion, and/or eye irritation during the JCP season, usually February through April, while none of the normal subjects expressed any of these symptoms. All the 16 patients, but none of the 9 normal subjects, showed positive results for JCP quantitative CAP-RAST-FEIA (Pharmacia, Uppsala, Sweden).

CULTURE MEDIUM AND REAGENTS

All cultures were conducted in medium RPMI 1640 (Life Technologies, Inc., Grand Island, NY, USA) supplemented with penicillin G (100 U/ml), streptomycin (100 μg/ml), L-glutamine (0.3 mg/ml), and 10% fetal bovine serum (Life Technologies). Cedar Pollen Extract-Cj (Japanese cedar pollen) was purchased from Cosmo Bio (Tokyo, Japan). 64.1, a murine IgG_2a mAb to the human CD3 molecular complex was a gift of Dr. Peter E. Lipsky, NIAMS, Bethesda, MD, USA. Recombinant human IL-4 and IL-5 were purchased from Pepro Tech EC (London, UK).

CELL PREPARATION AND PURIFICATION

Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation of heparinized venous blood over sodium diatrizoate-Ficoll gradients (Histopaque; Sigma Chemical Co., St. Louis, MO, USA). PBMC were depleted of monocytes and natural killer (NK) cells by incubation with 5 mM L-leucine methyl ester HCl (Sigma) in serum-free RPMI 1640, as described elsewhere.[10] The treated cell population was washed twice and then incubated with neuraminidase-treated sheep erythrocytes. The rosetting and non-rosetting populations were then separated by centrifugation on sodium diatrizoate-Ficoll gradients. The sedimented rosetting population was treated with isotonic NH_4Cl to lyse the sheep erythrocytes, and then was passed over a nylon-wool column to remove residual B cells. The T cell population contained <0.1% esterase-positive monocytes and <0.5% CD20 B cells. The non-rosetting cells obtained from the interface were again rosetted with neuraminidase-treated sheep erythrocytes and centrifuged on sodium diatrizoate-Ficoll gradients to remove residual T cells. The resultant population of B cells contained < 1% T cells as determined by staining with anti-CD3 and anti-CD2 pan T cell mAb, followed by analysis by flow cytometry. The cells were additionally characterized as containing >90% CD20-positive B cells, <1% CD14-positive monocytes, and no CD16-positive NK cells, as determined by flow cytometry.

CELL CULTURE TECHNIQUES OF FOR INDUCTION OF IG PRODUCTION

MAb 64.1 was diluted in RPMI 1640 (2 μg/ml), and 50 μl was placed in each well of 96-well flat bottom microtiter plates (No. 3596; Costar, Cambridge, MA, USA) and incubated at room temperature for 2 h. The wells were then washed once with culture medium to remove nonadherent mAb before the cells were added. Approximately 14–20% of the added mAb adhered to the wells.[11] Cultures were carried out in duplicated wells in a total volume of 200 μl. B cells (1×10^5/well) were cultured with autologous T cells that had been treated with mitomycin C before culture (2×10^5/well) in wells with immobilized anti-CD3, as previously described.[12] IL-4 (10 ng/ml) or IL-5 (10 ng/ml) was added where indicated. The cells were incubated for 10 days at 37℃ in a humidified atmosphere of 5% CO_2 and 95% air.

MEASUREMENT OF IGE, IGM AND IGG

The concentration of IgE, IgM and IgG was measured by ELISA. Briefly, microtiter plates (NUNC, Roskilde, Denmark) coated with $F(ab')_2$ fragments of goat anti-human IgM, IgG (Organon Teknika, Durham, NC, USA) or IgE (Chemicon, Temecula, CA, USA) were incubated with cell-free culture superna-

tants or IgM, IgG or IgE standards in PBS containing 1% bovine serum albumin (BSA; Miles, Elkhart, IN, USA). Bound Ig were detected with peroxidase-conjugated $F(ab')_2$ fragments of goat anti-human IgM or IgG (Organon Teknika) or IgE (Biosource, Camarillo, CA, USA) as previously described.[13]

MEASUREMENT OF ANTI-JCP ANTIBODIES

The concentration of anti-JCP antibodies were measured by ELISA. Briefly, wells of 96-well microtiter soft plates (Dynatech Lab, Alexandria, VA, USA) were coated with 50 µl solution of 10 µg protein/ml Cedar Pollen Extract-Cj (Cosmo Bio) in 0.05 M carbonate buffer, pH 9.6, at 4℃ for overnight, and then were overcoated with PBS containing 1% BSA at room temperature for 2 h. The wells were incubated with culture supernatants, serum samples (diluted in PBS containing 1% BSA by 1 : 20–200), or with standard sera serially diluted in PBS containing 1% BSA, for 3 h at room temperature. Bound Ig were detected with peroxidase conjugated $F(ab')_2$ fragments goat anti-human IgE, IgG or IgM diluted with PBS containing 1% BSA (anti-IgE; 1 : 5000, anti-IgG and IgM; 1 : 20000). Development was carried out with substrate solution containing o-phenylene diamine and H_2O_2, the reaction of which was stopped by 5N H_2SO_4, as previously described.[14] Determination of the absorbance at 492 nm (OD492) was normalized to standard sera such that anti-JCP antibodies might be converted to an arbitrary unit scale. The concentration of anti-JCP antibodies that produced half of the maximal OD492, given by saturating concentrations of anti-JCP in the ELISA plates, was arbitrary defined as 1 U/ml.[14] Nonspecific binding activities to wells without Cedar Pollen Extract-Cj were also determined in reference to the standard curves for binding activities to Cedar Pollen Extract-Cj. The specific anti-JCP activities were thus determined by subtracting the values for the nonspecific binding activity from those for the binding activity to Cedar Pollen Extract-Cj. The anti-JCP specific IgE levels in sera measured in our ELISA were closely correlated with those obtained by CAP-RAST-FEIA (Pharmacia, Uppsala, Sweden) ($p < 0.0001$, $r = 0.942$). The intraassay and interassay coefficients of variation in our ELISA for anti-JCP specific IgE were 7.1% and 13.8%, respectively.

RESULTS

SERUM ANTI-JCP ANTIBODIES IN PATIENTS WITH JAPANESE CEDAR POLLINOSIS AND IN NORMAL SUBJECTS

Initial experiments explored the serum levels of anti-JCP antibodies using ELISA developed by us in patients with Japanese cedar pollinosis. Anti-JCP antibodies were also evaluated by anti-JCP indices, calculated as anti-JCP antibodies of each isotype divided by total corresponding immunoglobulin isotype levels. As shown in Figure 1, there were no significant differences in serum IgE, IgG or IgM levels between pollinosis patients and normal subjects. In addition, there were no significant differences in anti-JCP specific IgM between normal subjects and pollinosis patients, whereas serum anti-JCP specific IgE as well as anti-JCP specific IgG were higher in pollinosis patients. Of note, IgE and IgG anti-JCP indices were higher in pollinosis patients than in normal subjects, whereas there was no significant difference in IgM anti-JCP indices between the 2 groups. These results are consistent with those obtained from CAP-RAST-FEIA (data not shown). Moreover, the data also suggest that antigen-specific IgG and IgE immune responses might take place in pollinosis patients.

PRODUCTION OF ANTI-JCP ANTIBODIES BY PERIPHERAL BLOOD B CELLS STIMULATED BY IMMOBILIZED ANTI-CD3 ACTIVATED T CELLS

Next experiments were carried out to explore whether peripheral blood B cells from pollinosis patients have higher capacity to produce anti-JCP antibodies. Highly purified peripheral blood B cells isolated from normal subjects and Japanese cedar pollinosis patients were incubated for 10 days with mitomycin C treated autologous T cells in the presence of immobilized anti-CD3. As shown in Figure 2, B cells from pollinosis patients produced higher amounts of anti-JCP specific IgE ($p = 0.0006$), but not anti-JCP specific IgG and IgM than those from normal subjects. Of note, B cells from pollinosis patients produced higher amounts of total IgE than those from normal subjects. However, IgE anti-JCP indices were higher in culture supernatants of pollinosis patients B cells than in those of normal subjects B cells. There were no significant differences in IgM and IgG anti-JCP indices in culture supernatants between the 2 groups. These results indicate that B cells from pollinosis patients have higher capacities to produce total IgE as well as anti-JCP specific IgE in response to anti-CD3 stimulated autologous T cells. Moreover, the data also suggested that within the IgE producing B cells of pollinosis patients the number of JCP-specific B cells might be increased.

TH2 CYTOKINES (IL-4, IL-5) OR T CELLS FROM JAPANESE CEDAR POLLINOSIS PATIENTS COULD NOT ENHANCE THE PRODUCTION OF ANTI-JCP SPECIFIC IGE BY B CELLS FROM NORMAL INDIVIDUALS

A number of studies have emphasized the role of T cells, especially Th2 cells, in the elucidation of IgE responses. It was therefore possible that the enhanced capacity of pollinosis patients B cells to produce IgE or anti-JCP specific IgE might result from the capacity of T cells to produce Th2 cytokines. To test this point, experiments were carried out in which B cells from normal healthy donors were cultured with mito-

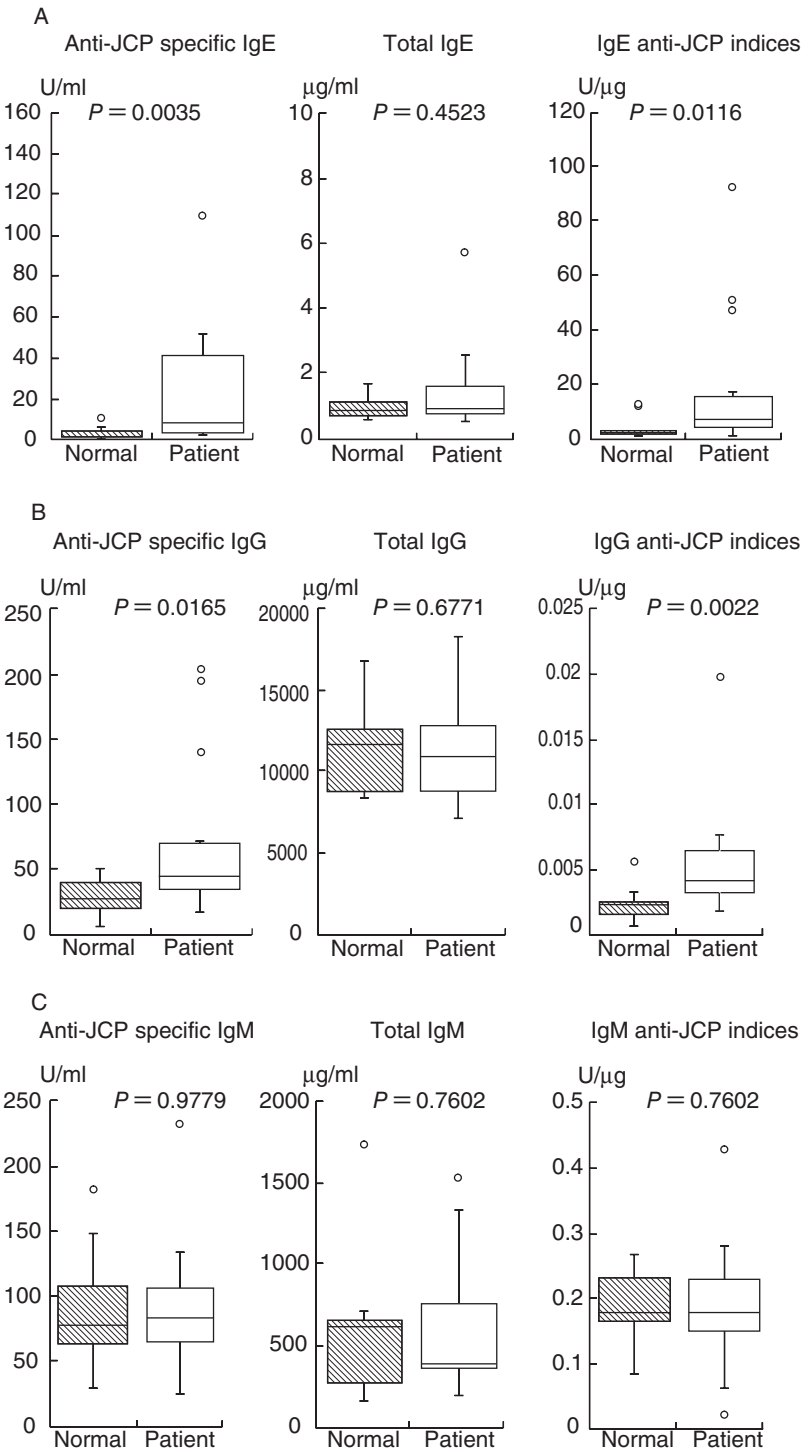

Fig. 1 Serum levels of IgE, IgG and IgM anti-Japanese cedar pollen (JCP) antibodies. Sera from 9 normal individuals and 16 patients with pollinosis were assayed for total IgE, IgG and IgM levels or anti-JCP specific IgE, IgG and IgM by ELISA. Anti-JCP indices were calculated by anti-JCP levels of each isotype (U/ml) divided by corresponding total Ig levels (μg/ml). Data are expressed in box plots, in which horizontal lines at the top, middle, and bottom of the boxes show the 75th, 50th, and 25th percentiles, respectively, and vertical lines above and below the boxes show the 90th and 10th percentiles, respectively. Statistical significance was evaluated by Mann-Whitney's *U* test.

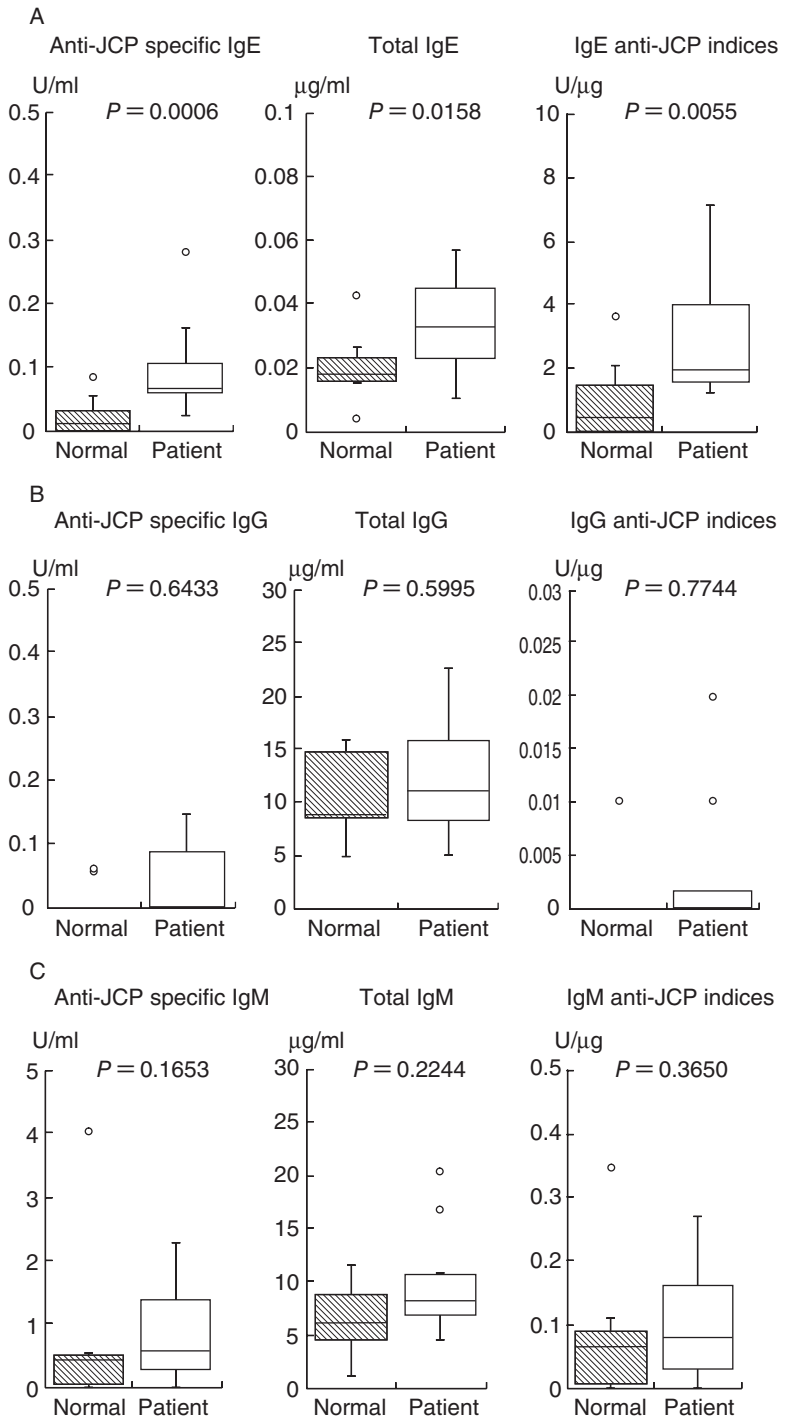

Fig. 2 Production of IgE, IgG and IgM anti-Japanese cedar pollen (JCP) antibodies by peripheral blood B cells. B cells (1×10^5/well) were cultured with mitomycin C treated T cells (2×10^5/well) in wells with immobilized anti-CD3 (64.1, 100 ng/well) . After 10 days from the initiation of the culture, the supernatants were harvested and assayed for total IgE, IgG and IgM levels or anti-JCP specific IgE, IgG and IgM by ELISA. Data are expressed in box plots, in which horizontal lines at the top, middle, and bottom of the boxes show the 75th, 50th, and 25th percentiles, respectively, and vertical lines above and below the boxes show the 90th and 10th percentiles, respectively. Statistical significance was evaluated by Mann-Whitney's *U* test.

Table 1 Effects of exogenous Th2 cytokines (IL-4 and IL-5) on the production of anti-JCP specific IgE and IgM.

Addition	Anti-JCP specific IgE (U/ml)	Total IgE (μg/ml)	IgE anti-JCP indices (U/μg)	Anti-JCP specific IgM (U/ml)	Total IgM (μg/ml)	IgM anti-JCP indices (U/μg)
Nil	0.047 ± 0.016	0.020 ± 0.003	2.304 ± 0.640	1.636 ± 1.191	8.362 ± 1.737	0.159 ± 0.094
IL-4	0.036 ± 0.009	0.026 ± 0.006	1.474 ± 0.453	1.012 ± 0.616	7.488 ± 1.025	0.121 ± 0.062
IL-5	0.043 ± 0.013	0.025 ± 0.003	1.664 ± 0.412	1.763 ± 1.262	8.602 ± 0.875	0.182 ± 0.117
IL-4/IL-5	0.060 ± 0.029	0.024 ± 0.001	2.533 ± 1.268	1.030 ± 0.653	7.663 ± 0.929	0.119 ± 0.064

Normal B cells (1×10^5/well) were cultured with mitomycin C treated normal T cells (2×10^5/well) in wells with immobilized anti-CD3 (64.1, 100 ng/well) in the presence or absence of IL-4 (10 ng/ml) or IL-5 (10 ng/ml). After 10 days from the initiation of the culture, the supernatants were harvested and assayed for total IgE and IgM levels or anti-JCP specific IgE and IgM by ELISA. Mean ± SD values of 3 different experiments are presented.

mycin C treated autologous T cells in wells with immobilized anti-CD3 in the presence or absence of IL-4 and IL-5. As shown in Table 1, addition of IL-4 and IL-5 did not enhance the production of anti-JCP specific IgE or total IgE by B cells from normal subjects. Accordingly, addition of IL-4 and IL-5 did not significantly increase the IgE anti-JCP indices in the culture supernatants.

Next experiments examined whether T cells from pollinosis patients might enhance the production of IgE or anti-JCP specific IgE by B cells from normal healthy subjects. B cells from normal subjects were cultured with mitomycin-C-treated T cells from patients with cedar pollinosis in wells with immobilized anti-CD3. However, T cells from pollinosis patients did not enhance the production of anti-JCP specific IgE or total IgE or IgE anti-JCP indices by B cells from normal subjects (Fig. 3). These results indicate that the enhanced capacity of pollinosis patients B cells to produce anti-JCP specific IgE are due to the abnormalities of B cell population, but not those of T cell population. Since there was no significant difference in the capacity of B cells to produce anti-JCP specific IgM between pollinosis patients and normal subjects, it is suggested that pollinosis patients have a larger number of B cells that had undergone class-switch to be committed to produce anti-JCP specific IgE.

DISCUSSION

In the present study, we have developed ELISA for measurement of anti-JCP antibodies of IgM, IgG and IgE isotypes. Anti-JCP specific IgE determined by this ELISA correlated well with CAP-RAST-FEIA, confirming the reliability of this ELISA. Serum anti-JCP specific IgE were significantly higher in pollinosis patients than in normal subjects. In addition, the ratio of anti-JCP specific IgE to total IgE (IgE anti-JCP indices) was significantly elevated in pollinosis patients, confirming that Cj antigen-specific IgE responses take place in pollinosis patients. There was no difference in the binding capacity of anti-JCP specific IgE between normal individuals and pollinosis patients. Thus, binding curves generated by serially diluted sera from normal individuals (OD492 values versus dilution) were parallel to those generated by serially diluted sera from pollinosis patients (data not shown). It is therefore suggested that the affinity maturation of IgE to JCP was comparable between normal individuals and pollinosis patients.

By contrast, there were no significant differences in anti-JCP specific IgM or IgM anti-JCP indices between pollinosis patients and normal healthy individuals, suggesting that there might be comparable numbers of B cells producing anti-JCP specific IgM in the peripheral blood of normal healthy individuals. Of note, serum anti-JCP specific IgG and IgG anti-JCP indices were significantly elevated in pollinosis patients compared with normal individuals. The data suggest that anti-JCP producing B cells undergo class switch to IgG as well as to IgE in pollinosis patients, as is consistent with previous observations suggesting the sequential switching from mu to epsilon via gamma in human B cells.[15-17]

The results in the current studies also demonstrated that peripheral blood B cells from normal individuals produce comparable amounts of anti-JCP specific IgM to those from pollinosis patients upon stimulation with immobilized anti-CD3 stimulated autologous T cells. Moreover, IgM anti-JCP indices in the culture supernatants of normal B cells were also comparable to those in the culture supernatants of pollinosis patients B cells. The data therefore confirm that there are comparable numbers of B cells producing anti-JCP specific IgM in the peripheral blood of normal individuals to that of pollinosis patients. By contrast, peripheral blood B cells from pollinosis patients produced higher amounts of anti-JCP specific IgE than those from normal individuals. Since IgE anti-JCP indices were also higher in the culture supernatants of B cells from pollinosis patients, it is suggested that the clone size of anti-JCP antibody producing B cells might be expanded within IgE producing B cells in pollinosis patients.

Shinmoto and colleagues explored the capacity of peripheral blood B cells from cedar pollinosis pa-

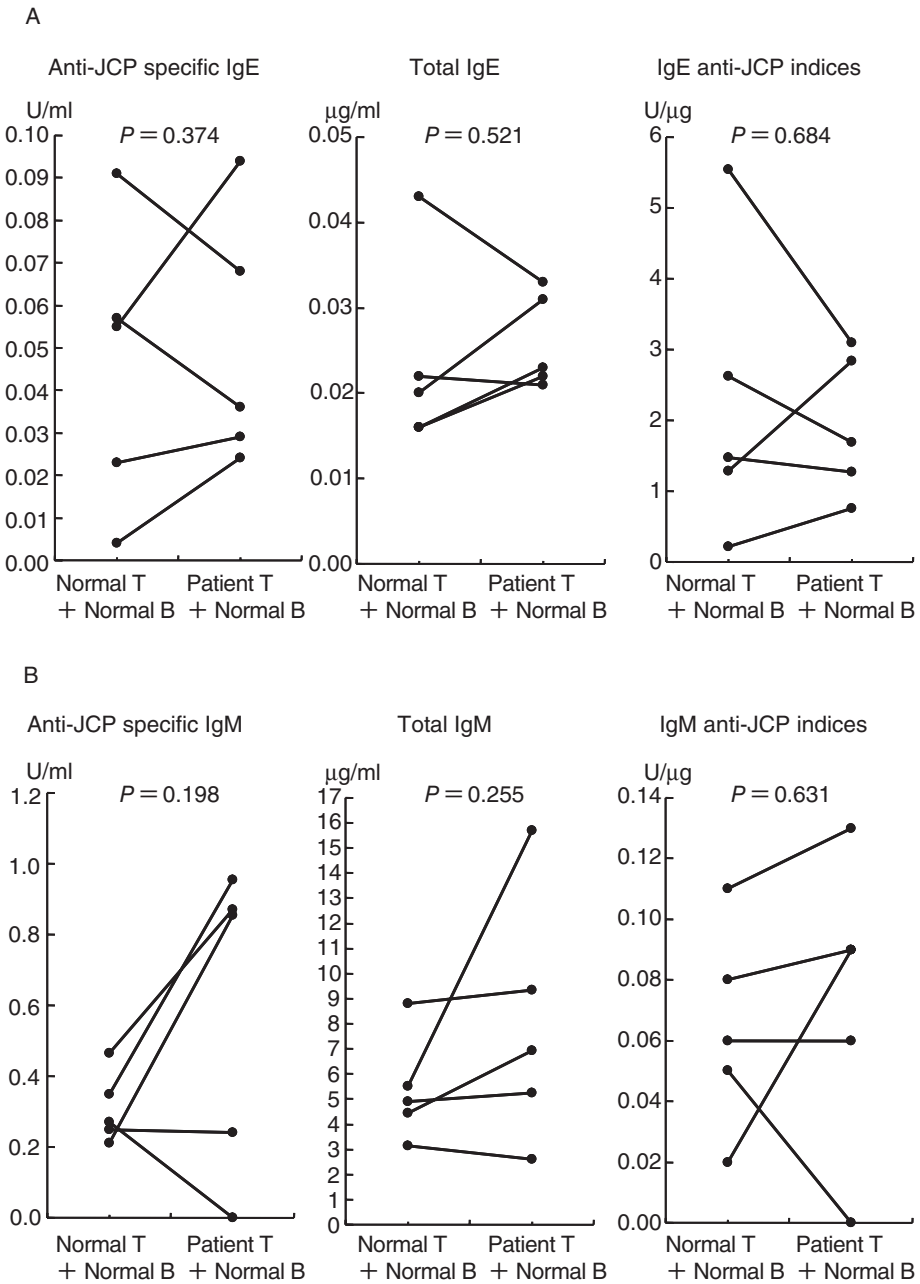

Fig. 3 T cells from pollinosis patients do not enhance the production of anti-JCP specific IgE by B cells from healthy donors. B cells (1×10^5/well) from 5 different healthy donors were cultured with mitomycin C treated autologous T cells or T cells (2×10^5/well) from one of 5 different pollinosis patients in wells with immobilized anti-CD3 (64.1, 100 ng/well). After 10 days from the initiation of the culture, the supernatants were harvested and assayed for total IgE and IgM levels or anti-JCP specific IgE and IgM by ELISA. Statistical significance was evaluated by paired sample *t* test.

tients to produce antibodies to pollen antigens using EB virus. Most of the transformed B cells produced IgM antibodies to pollen antigens, whereas there were no transformed B cells which produced IgE antibodies to pollen antigens.[7] There were no differences in the frequency to B cells to be transformed to produce IgM antibodies against pollen antigen between healthy individuals and pollinosis patients.[7] Their results are consistent with our observation that there are comparable numbers of B cells producing anti-JCP specific IgM in the peripheral blood of normal individuals to that of pollinosis patients. It should

be pointed out, however, that EB virus was not potent enough to activate B cells that are committed to produce anti-JCP specific IgE. In this regard, previous studies demonstrated that the precursor frequency of IgM producing B cells activated by EB virus was approximately 0.027–0.34/100 B cells, which appeared to be approximately 10% of that induced by immobilized anti-CD3 stimulated $CD4^+$ T cells (0.84–4.65/100 B cells).[9] Thus, in the present study, immobilized anti-CD3 activated T cells could induce the production of anti-JCP specific IgE by B cells in pollinosis patients, but not in healthy individuals. These results therefore suggest that the clone size of B cells that produce anti-JCP specific IgE might be too small to be activated by EB virus even in pollinosis patients. More importantly, our system utilizing immobilized anti-CD3 might be a powerful tool to study the regulatory mechanism of production of anti-JCP specific IgE, disclosing the differences in the capacity to produce anti-JCP specific IgE by peripheral blood B cells between pollinosis patients and normal individuals.

In our system with immobilized anti-CD3, direct interactions between CD154 on activated T cells and CD40 on B cells, as well as cytokines, including IL-2 and IL-4, have been shown to play crucial roles in B cell differentiation.[12,18-20] It should be pointed out that an analysis using limiting dilution is necessary to calculate the number of antibody-producing cells. Of note, the results in the previous study disclosed that the amounts of autoantibodies, such as rheumatoid factor and anti-DNA paralleled the precursor frequencies of such autoantibody producing B cells in our system with immobilized anti-CD3.[21] Therefore, it is also likely that the number of IgE producing B cells might be increased, although the direct demonstration with limiting dilution analysis would be required.

Although serum anti-JCP specific IgG were higher in pollinosis patients than in normal individuals, there were no significant differences in the capacity of peripheral blood B cells to produce anti-JCP specific IgG between pollinosis patients and normal individuals. This might be accounted for by the fact that the clone size of IgG anti-JCP producing B cells within IgG producing B cells is too small, reflected by extremely lowever IgG anti-JCP indices compared with IgE anti-JCP indices or IgM anti-JCP indices in the sera as well as in the culture supernatants. It was thus likely that even immobilized anti-CD3 stimulated T cells could not elicit sufficient amounts of anti-JCP specific IgG to be compared.

The results from experiments of activation of B cells by immobilized anti-CD3 stimulated T cells confirm that anti-JCP producing B cells undergo class-switch to IgE in pollinosis patients. A number of studies have disclosed that Th2 cytokines, especially IL-4, and direct cellular interactions between T cells and B cells might be necessary for IgE isotype switching.[5,6] However, exogenous IL-4 and IL-5 did not elicit the production of anti-JCP specific IgE by normal B cells. Moreover, T cells from pollinosis patients could not induce the production of anti-JCP specific IgE from normal B cells upon stimulation with immobilized anti-CD3. Taken together, the results indicate that exogenous Th2 cytokines and activated T cells from pollinosis patients would not be sufficient for IgE isotype class switching of IgM anti-JCP producing B cells. Steinberger and colleagues showed that allergen-specific IgE producing B cells are not responsive to IL-4 mediated signals.[22] Thus, IL-4 and anti-IL-4 affected the in vitro production of total IgE, but not that of allergen-specific IgE, by peripheral blood B cells from pollinosis patients.[22] Accordingly, Mitchell and colleagues also demonstrated that IL-4 promotes polyclonal B cell activation in the in vitro system with immobilized anti-CD3.[20] Taken together, these results may have negative implications for attempts to modulate allergen-specific IgE production in pollinosis patients with IL-4 antagonists. Of note, recent studies have emphasized the role of direct cellular interactions between accessory cells, including dendritic cells, and B cells that lead to immunoglobulin class switching under certain circumstances.[23-25] Further studies to explore the role of such interactions in the IgE isotype class switching would be required for a complete understanding of the mechanism of the IgE production.

REFERENCES

1. Horiguchi S, Saito Y. Discovery of Japanese Cedar Pollinosis in Nikko, Ibaraki Prefecture. *Arerugi* 1964;**13**:16-18.
2. Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* 1999;**402**:B24-30.
3. Stingl G, Maurer D. IgE-mediated allergen presentation via Fc epsilon RI on antigen-presenting cells. *Int. Arch. Allergy Immunol.* 1997;**113**:24-29.
4. Bieber T. Fc epsilon RI-expressing antigen-presenting cells: new players in the atopic game. *Immunol. Today* 1997;**18**:311-313.
5. Oettgen HC. Regulation of the IgE isotype switch: new insights on cytokine signals and the functions of epsilon germline transcripts. *Curr. Opin. Immunol.* 2000;**12**:618-623.
6. Bacharier LB, Geha RS. Molecular mechanisms of IgE regulation. *J. Allergy Clin. Immunol.* 2000;**105**:S547-558.
7. Shinmoto H, Takahashi T, Shinmoto M. Human antibodies to cedar pollen secreted by human peripheral lymphocytes transformed by Epstein-Barr virus. *Arerugi* 2004; **53**:14-18.
8. Yarchoan R, Tosato G, Blaese RM, Simon RM, Nelson DL. Limiting dilution analysis of Epstein-Barr virus-induced immunoglobulin production by human B cells. *J. Exp. Med.* 1983;**157**:1-14.
9. Amoroso K, Lipsky PE. Frequency of human B cells that differentiate in response to anti-CD3-activated T cells. *J. Immunol.* 1990;**145**:3155-3161.
10. Thiele DL, Kurosaka M, Lipsky PE. Phenotype of the accessory cell necessary for mitogen-stimulated T and B cell responses in human peripheral blood: delineation by its sensitivity to the lysosomotropic agent, L-leucine

methyl ester. *J. Immunol.* 1983;**131**:2282-2290.
11. Geppert TD, Lipsky PE. Accessory cell independent proliferation of human T4 cells stimulated by immobilized monoclonal antibodies to CD3. *J. Immunol.* 1987;**138**: 1660-1666.
12. Hirohata S, Jelinek DF, Lipsky PE. T cell-dependent activation of B cell proliferation and differentiation by immobilized monoclonal antibodies to CD3. *J. Immunol.* 1988; **140**:3736-3744.
13. Hirohata S, Yamada A, Inoue T. A sensitive and simple method for determination of IgM in cerebrospinal fluid by a solid-phase enzyme-immunoassay. Comparison of two different methods. *J. Neurol. Sci.* 1985;**67**:115-118.
14. Oka H, Hirohata S, Inoue T, Iwamoto S, Miyamoto T. Quantitative determination of IgM-rheumatoid factor by enzyme immunoassay—standardization using a serum from a rheumatoid arthritis patient. *Clin. Chim. Acta* 1990;**188**:147-159.
15. Fujieda S, Zhang K, Saxon A. IL-4 plus CD40 monoclonal antibody induces human B cells gamma subclass-specific isotype switch: switching to gamma 1, gamma 3, and gamma 4, but not gamma 2. *J. Immunol.* 1995;**155**:2318-2328.
16. Loh RK, Jabara HH, Geha RS. Disodium cromoglycate inhibits S mu— > S epsilon deletional switch recombination and IgE synthesis in human B cells. *J. Exp. Med.* 1994; **180**:663-671.
17. Jabara HH, Loh R, Ramesh N, Vercelli D, Geha RS. Sequential switching from mu to epsilon via gamma 4 in human B cells stimulated with IL-4 and hydrocortisone. *J. Immunol.* 1993;**151**:4528-4533.
18. Hirohata S. The role of CD40-CD40 ligand interactions in suppression of human B cell responsiveness by CD4+ T cells. *Cell. Immunol.* 1997;**182**:20-28.
19. Nishioka Y, Lipsky PE. The role of CD40-CD40 ligand interaction in human T cell-B cell collaboration. *J. Immunol.* 1994;**153**:1027-1036.
20. Mitchell LC, Davis LS, Lipsky PE. Promotion of human T lymphocyte proliferation by IL-4. *J. Immunol.* 1989;**142**: 1548-1557.
21. Hirohata S, Inoue T, Miyamoto T. Frequency analysis of human peripheral blood B cells producing IgM-rheumatoid factor. Differential effects of stimulation with monoclonal antibodies to CD3 and Staphylococcus aureus. *J. Immunol.* 1990;**145**:1681-1686.
22. Steinberger P, Bohle B, di Padova F *et al*. Allergen-specific IgE production of committed B cells from allergic patients *in vitro*. *J. Allergy Clin. Immunol.* 1995;**96**:209-218.
23. Fayette J, Dubois B, Vandenabeele S *et al*. Human dendritic cells skew isotype switching of CD40-activated naive B cells towards IgA1 and IgA2. *J. Exp. Med.* 1997;**185**: 1909-1918.
24. Litinskiy MB, Nardelli B, Hilbert DM *et al*. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat. Immunol.* 2002;**3**:822-829.
25. Hirohata S, Yanagida T, Nagai T *et al*. Bone marrow CD34+ progenitor cells stimulated with stem cell factor and GM-CSF have the capacity to activate IgD-B cells through direct cellular interaction. *J. Leukoc. Biol.* 2002; **71**:987-995.