

Allergen-Induced Synthesis of Interleukin-5, but not of IgE, is a Key Mechanism Linked to Symptomatic Episodes of Seasonal Allergic Rhinitis in Sensitized Individuals

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Some individuals with detectable levels of Japanese cedar (*Cryptomeria japonica*) pollen-specific immunoglobulin (Ig)E in serum have no apparent nasal symptoms during the pollen season. The response of CD4⁺ T-helper (Th) cells to the pollen allergen might differ fundamentally between asymptomatic and symptomatic individuals who are already sensitized to the pollen. The aim of this study was to discern the possible differences in responses of peripheral blood mononuclear cells (PBMCs) to the pollen allergen between asymptomatic and symptomatic subjects who have been sensitized to the pollen. This study included 20 non-atopic healthy volunteers (non-atopic group) and 48 patients who had detectable levels of the pollen-specific IgE before the pollen season in 1997. In the review of nasal symptoms during the pollen season 1997, 24 patients had typical symptoms of seasonal allergic rhinitis (symptomatic group), and the remainder had no seasonal aggravation of nasal symptoms (asymptomatic group). Peripheral blood mononuclear cells (1.0×10^7 cells/well) were obtained from each individual during the pollen season and cultured in the absence or presence of 12.5 µg of Cry j 1 for 4 days. The concentrations of IgE, interleukin-5 (IL-5), and interferon-gamma (IFN-γ) in the culture supernatants were measured. The levels of IgE produced by Cry j 1-stimulated PBMCs of the asymptomatic and symptomatic groups were significantly higher than those of the non-atopic group, but did not differ between the asymptomatic and symptomatic groups. The levels of IL-5 produced by Cry j 1-stimulated PBMCs did not differ significantly between the non-atopic group and the asymptomatic group, but the levels of IL-5 were significantly higher in the symptomatic group than in the asymptomatic group as well as the non-atopic group. The levels of IFN-γ produced by Cry j-1 stimulated PBMCs did not differ significantly among the three groups. In conclusion, our study has suggested that Japanese cedar pollen-induced synthesis of IL-5, but not of IgE or IFN-γ, is likely to be a key mechanism linked to the symptomatic episode of seasonal allergic rhinitis in individuals sensitized to the pollen.

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INTRODUCTION

Japanese cedar (*Cryptomeria japonica*) pollinates heavily during early spring and is the most important allergen causing seasonal allergic rhinitis in Japan. The incidence of patients is estimated to be over 10% of the population. One of the key

factors involved in seasonal allergic rhinitis due to cedar pollen is undoubtedly sensitization to the cedar pollen or having the pollen-specific immunoglobulin (Ig)E. However, the detection of specific IgE does not always coincide with clinical allergic manifestation, but merely indicates acquired sensitization to the pollen or a marker of possible future allergic manifestation.

Indeed, some individuals with detectable levels of specific IgE to the pollen in serum have no apparent nasal symptoms during the pollen season. It is of key importance to elucidate the determinant underlying difference between asymptomatic and symptomatic individuals who have already been sensitized to the pollen.

There is increasing evidence that airway allergic diseases represent a specialized form of cell-mediated immunity, in which CD4⁺ T-helper (Th) cells and their products, various cytokines, play a central part in the orchestration and persistence of airway allergic inflammation. Cytokines derived from Th2 cells could mediate allergic inflammation by activating eosinophils, promoting mast cell development, regulating immunoglobulin isotypes switching to IgE, and modulating adhesion molecule expression. Currently allergy might be defined as a Th2-like cell or Th2 response-predominant inflammatory disease [1]. The response of Th cells to the allergen might differ fundamentally between asymptomatic and symptomatic individuals, and the difference might underlie the different clinical manifestation between them. The aim of this study was thus to discern the possible differences in responses of peripheral blood mononuclear cells (PBMCs) to the pollen allergen between asymptomatic and symptomatic subjects who have already been sensitized to the pollen.

MATERIALS AND METHODS

Patients. This study included 48 patients and 20 non-atopic healthy volunteers who gave informed consent for participation. All the patients were selected from our outpatients who had detectable levels of specific IgE for Japanese cedar (*Cryptomeria japonica*) pollen before the pollen season (January) in 1997. In the review of nasal symptoms during the pollen season 1997, 24 had typical symptoms of seasonal allergic rhinitis (12 females and 12 males, age range 18–40 years, symptomatic group), and the remaining 24 had no seasonal aggravation of nasal symptoms (12 females and 12 males, age range 20–36 years, asymptomatic group). Twenty non-atopic healthy volunteers (10 females and 10 males, age range 18–38 years, non-atopic group), matched for age and sex, were chosen on the basis of the following criteria: (1) no history of allergic disease; (2) no physical findings indicative of atopic disease; and (3) negative serum IgE antibodies specific to the major allergens in Japan, such as house dust mites, Japanese cedar pollen, ragweed, Japanese cypress pollen, and molds.

Study design. This study design followed the principles outlined in the Declaration of Helsinki [2]. Pollination of Japanese cedars in 1997 occurred from the second week of February to the third week of April. Peripheral blood was collected from each subject during the cedar pollen season (first week of April in 1997), and serum concentrations of pollen-specific IgE and total IgE were determined by the use of the lumiward immunoassay system [3]. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples by Ficoll–density gradient centrifugation. The PBMCs were washed twice in phosphate-buffered saline (PBS) and resuspended in RPMI-1640 (Flow Laboratories, Meckenheim, Germany) containing 10% inactivated fetal calf serum (FCS: JRH Biosciences, Lenexa, CA, USA), 100 µg/ml streptomycin (Sigma, St Louis, MO, USA), 100 IU/ml penicillin and 2 mM L-glutamine. Then PBMCs from each subject were cultured at a final concentration of 1.0×10^7 cells/well in 12-well plates (Iwaki Glass, Tokyo, Japan) at 37°C in a fully humidified 5% CO₂ atmosphere.

To observe allergen-induced synthesis of IgE and cytokines, PBMCs from 10 subjects in the non-atopic group, 14 patients in the asymptomatic group and 14 patients in the symptomatic group were stimulated with 12.5 µg of Cry j 1 for 4 days. To observe spontaneous synthesis of IgE and cytokines, PBMCs from each set of 10 subjects in the three groups were cultured for 4 days in the absence of Cry j 1. Then the culture supernatants were harvested by centrifugation. All the culture supernatants were stored at –80°C until measurement of IgE, interleukin-5 (IL-5), and interferon-gamma (IFN-γ).

Determination of IgE, IL-5, and IFN-γ in culture supernatants. Determination of IgE was by the use of the lumiward immunoassay system [3] while IL-5 and IFN-γ were measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available kits. The details and justification of the methods employed in this study have been described elsewhere [4–6].

In brief, IL-5 was measured by an ELISA, using an ELISA kit (Otsuka Pharmaceutical Co. Ltd, Tokushima, Japan). Microtitration plates were coated with anti-IL-5 monoclonal antibody (MoAb JES1-39D10 at 2 µg/ml) in 100 µl/well of PBS, pH 7.4. Following overnight incubation at 4°C, the wells were blocked with a 1% solution of skim milk in PBS for at least 1 h at room temperature and washed three times with PBS containing 0.05% Tween-20 (Tween-20–PBS: washing was always performed with this buffer). Then 100 µl of samples or standard in 0.1% bovine serum albumin (BSA)–PBS were added to the wells and the plates were incubated at 4°C for 24 h. The plates were washed three times, 100 µl of rabbit IL-5 antibodies (MoAb JES1-5A10: diluted to 1:500 with 0.1% BSA–PBS–Tween-20) were added to each well, and the plates were incubated for 2 h at 23°C. Subsequently, the plates were washed, supplemented with 100 µl of horseradish peroxidase (HRP)-labelled Avidin (diluted to 1:10 000 with 0.1% BSA–PBS–Tween-20) and incubated at 23°C for 2 h. Finally, 100 µl of enzyme substrate (1 mg/ml O-phenylene diamine and 0.03% H₂O₂ in 0.1 M sodium citrate buffer, pH 5.0) were added to each well and the plates were incubated at room temperature for 10 min. The reaction was stopped by adding 100 µl of H₂O₂ to each well and the absorbance at 492 nm was determined using a Titertek Multiscan. The lowest sensitivity of the method employed is 10 pg/ml of IL-5, and does not measure the presence of other cytokines.

An ELISA kit (Otsuka Pharmaceutical Co. Ltd) was used to determine IFN-γ. Microtitration plates were coated with anti-IFN-γ. Microtitration plates were coated with anti-IFN-γ monoclonal antibody (MoAb ANOC 1611 at 10 µg/ml) in 100 µl/well of PBS, pH 7.4. Following overnight incubation at 4°C, the wells were blocked with a 1% solution of skim milk in PBS for at least 1 h at room temperature and washed three times with PBS containing 0.05% Tween-20 (Tween-20–PBS: washing was always performed with this buffer). Then 100 µl of samples or standard in 0.1% BSA–PBS were added to the wells and the plates were incubated at 4°C for 24 h. The plates were then washed three times, 100 µl of rabbit anti-IFN-γ antibodies (OCT 1601: diluted to 1:2000 with 0.1% BSA–PBS–Tween-20) were added to each well, and the plates were incubated for 2 h at 23°C. Subsequently, the plates were washed, supplemented with 100 µl of HRP-labelled goat anti-rabbit IgG (diluted to 1:10 000 with 0.1% BSA–PBS–Tween-20) and incubated at 23°C for 2 h. Finally, 100 µl of enzyme substrate (1 mg/ml O-phenylene diamine and 0.03% H₂O₂ in 0.1 M sodium buffer, pH 5.0) were added to each well and the plates were incubated at room temperature for 10 min. The reaction was stopped by adding 100 µl of H₂SO₄ to each well and the absorbance at 492 nm was determined using a Titertek Multiscan. The lowest sensitivity of the method employed is 20 pg/ml of IFN-γ, and does not measure the presence of other cytokines.

Table 1. Serum levels of total IgE and specific IgE

	Total IgE (IU/ml)				Specific IgE (IU/ml)			
	Mean	SD	SEM	Range	Mean	SD	SEM	Range
Non-atopic group (<i>n</i> = 10)	196.6	120.1	38.0	38.0–412.0	—	—	—	—
Asymptomatic group (<i>n</i> = 10)	350.2	248.3	78.5	39.0–856.0	19.8	19.3	6.1	2.1–63.0
Symptomatic group (<i>n</i> = 10)	277.3	235.7	74.5	41.0–765.0	37.2	31.0	9.8	2.7–93.0

Statistical analysis. If the levels of IgE, IL-5 or IFN- γ in particular patients were below the sensitivity of the respective assay, the lowest detectable value (IgE, 0.06 IU/ml; IL-5, 10 pg/ml; IFN- γ , 20 pg/ml) obtained for that assay arbitrarily used for the present statistical analysis. For comparisons between different groups, the Mann–Whitney's *U*-test was used.

RESULTS

Japanese cedar pollen-specific IgE and total IgE in serum in the subjects used for the experiment to observe spontaneous synthesis of IgE, IL-5 and IFN- γ

The levels of total IgE in serum did not differ among the three groups used in the experiment to observe spontaneous synthesis of IgE and cytokines (non-atopic groups versus asymptomatic group, $P = 0.1618$; non-atopic group versus symptomatic group, $P = 0.6501$; asymptomatic group versus symptomatic group, $P = 0.4057$): see Table 1. Specific IgE was not detected in any individual in the non-atopic group. The levels of specific IgE in serum did not differ significantly between the asymptomatic and symptomatic groups ($P = 0.1736$).

Japanese cedar pollen-specific IgE and total IgE in serum in the subjects used for the experiment to observe allergen-stimulated synthesis of IgE, IL-5 and IFN- γ

The levels of total IgE in serum did not differ among the three groups (non-atopic group versus asymptomatic group, $P = 0.7508$; non-atopic group versus symptomatic group, $P = 0.5254$; asymptomatic group versus symptomatic group, $P = 0.2322$): see Table 2. Specific IgE was not detected in any individual in the non-atopic group. The

levels of specific IgE in serum did not differ significantly between the asymptomatic and symptomatic groups ($P = 0.0695$).

IgE, IL-5 and IFN- γ in culture supernatants

IgE concentration in culture supernatants. Spontaneous IgE synthesis was below the sensitivity in nine cultures from the non-atopic group, in eight cultures from the asymptomatic group, and eight cultures from the symptomatic group. The levels of spontaneous IgE synthesis were 0.06 ± 0.06 IU/ml (range 0.06–0.80 IU/ml) in the non-atopic group, 0.16 ± 0.27 IU/ml (range 0.06–0.90 IU/ml) in the asymptomatic group and 0.10 ± 0.09 IU/ml (range 0.06–0.31 IU/ml) in the symptomatic group, respectively.

The levels of IgE produced by Cry j 1-stimulated PBMCs were 0.38 ± 0.46 IU/ml in the non-atopic group, 1.27 ± 0.96 IU/ml in the asymptomatic group and 1.84 ± 2.4 IU/ml in the symptomatic group, respectively (Fig. 1). The levels of spontaneous IgE synthesis were not significantly different from those of Cry j 1-induced IgE synthesis in the non-atopic group ($P = 0.0912$). By contrast, the levels of Cry j 1-induced IgE synthesis were significantly higher than the levels of spontaneous IgE synthesis in the asymptomatic group ($P = 0.0021$) and the symptomatic group ($P = 0.0001$). The levels of IgE produced by Cry j 1-stimulated PBMCs in the asymptomatic group ($P = 0.0174$) as well as in the symptomatic group ($P = 0.0033$) were significantly higher than those in the non-atopic group. However, the levels of IgE produced by Cry j 1-stimulated PBMCs did not differ significantly between the asymptomatic and symptomatic groups ($P = 0.7822$).

IL-5 concentration in culture supernatants. Spontaneous IL-5 synthesis was below the sensitivity in every culture from the

Table 2. Serum levels of total IgE and specific IgE

	Total IgE (IU/ml)				Specific IgE (IU/ml)			
	Mean	SD	SEM	Range	Mean	SD	SEM	Range
Non-atopic group (<i>n</i> = 10)	262.6	133.2	94.2	168.4–356.8	—	—	—	—
Asymptomatic group (<i>n</i> = 14)	450.8	422.4	112.4	18.8–1268.0	16.9	14.7	3.9	1.3–52.0
Symptomatic group (<i>n</i> = 14)	338.2	451.4	120.7	29.0–1649.0	34.9	30.6	8.2	2.5–100.0

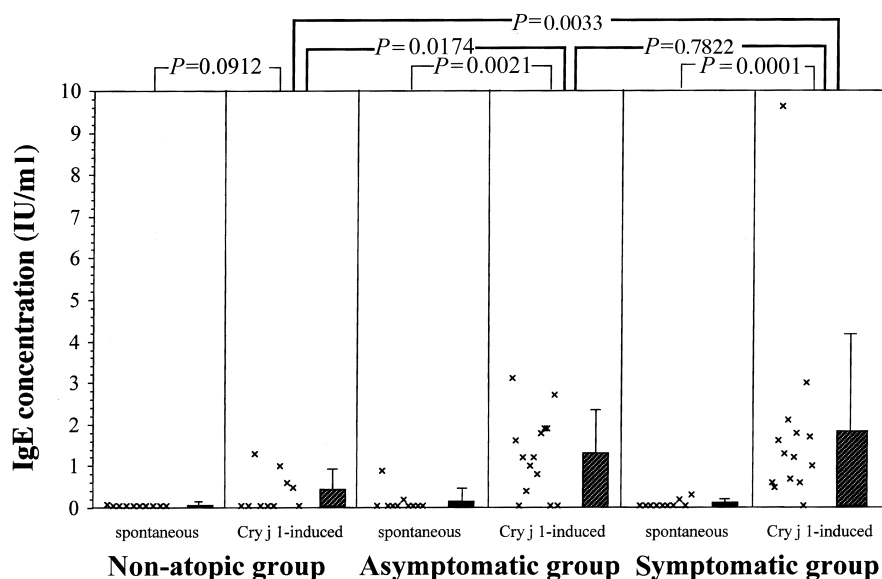


Fig. 1. The level of IgE produced in the culture supernatants. The level of IgE produced in the presence of Cry j 1 was not significantly different from the level of IgE produced in the absence of Cry j 1 in the non-atopic group. In the asymptomatic and symptomatic groups, the levels of IgE produced by PBMCs were significantly higher in the presence of Cry j 1 than in the absence of Cry j 1. The levels of IgE produced by Cry j 1-stimulated PBMCs in the asymptomatic group ($P=0.0174$) and the symptomatic group ($P=0.0033$) were significantly higher than those in the non-atopic group. However, the levels of IgE produced by Cry j 1-stimulated PBMCs did not differ significantly between the asymptomatic group and the symptomatic group ($P=0.7822$).

three groups. Concentrations of IL-5 in Cry j 1-stimulated culture supernatants were 50.7 ± 92.5 pg/ml in the non-atopic group, 50.8 ± 61.4 pg/ml in the asymptomatic group and 1052.7 ± 967.0 pg/ml in the symptomatic group, respectively (Fig. 2). The levels of Cry j 1-induced IL-5 synthesis were significantly higher than the levels of spontaneous IL-5 synthesis in each group (non-atopic group $P=0.0006$; asymptomatic group $P=0.0107$; symptomatic group $P<0.0001$). The levels of IL-5 produced by Cry j 1-stimulated PBMCs did not differ significantly between the non-atopic group and the asymptomatic group ($P=0.7636$). The levels of IL-5 produced by Cry j 1-stimulated PBMCs in the symptomatic group were significantly higher than those in the non-atopic group ($P<0.0001$) and those in the symptomatic group ($P<0.0001$).

IFN- γ concentration in culture supernatants. Spontaneous IFN- γ synthesis was below the sensitivity in all 10 cultures from the symptomatic group. Spontaneous IFN- γ synthesis was also below the sensitivity in nine cultures from the non-atopic group and nine from the asymptomatic group. The levels of spontaneous IFN- γ synthesis were 22.5 ± 7.9 IU/ml (range 20–45 pg/ml) in the non-atopic group and 22.2 ± 5.0 IU/ml (range 20–35 pg/ml) in the symptomatic group, respectively.

The levels of IFN- γ produced by Cry j 1-stimulated PBMCs were 133.3 ± 124.6 pg/ml in the non-atopic group, 180.9 ± 352.8 pg/ml in the asymptomatic group and 432.6 ± 823.7 pg/ml in the symptomatic group, respectively (Fig. 3). The levels of IFN- γ produced by Cry j 1-stimulated PBMCs were significantly higher than the levels of spontaneous

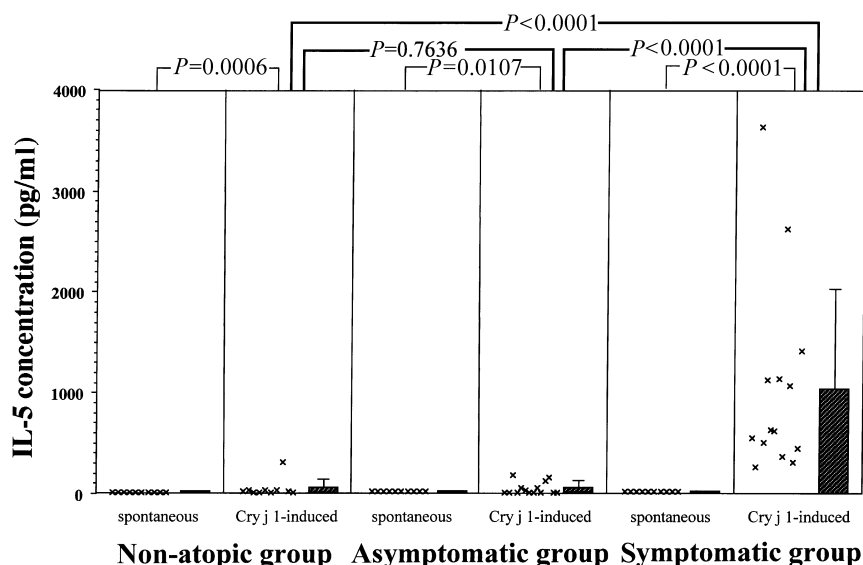


Fig. 2. The level of IL-5 produced in the culture supernatants. No PBMCs from any subject produced IL-5 above the minimal sensitivity (10 pg/ml) in the absence of Cry j 1. The levels of IL-5 produced by Cry j 1-stimulated PBMCs were significantly higher in the non-atopic group ($P<0.0001$) or the asymptomatic group ($P<0.0001$). On the other hand, the levels of IL-5 produced by Cry j 1-stimulated PBMCs did not differ significantly between the non-atopic group and the asymptomatic group ($P=0.7636$).

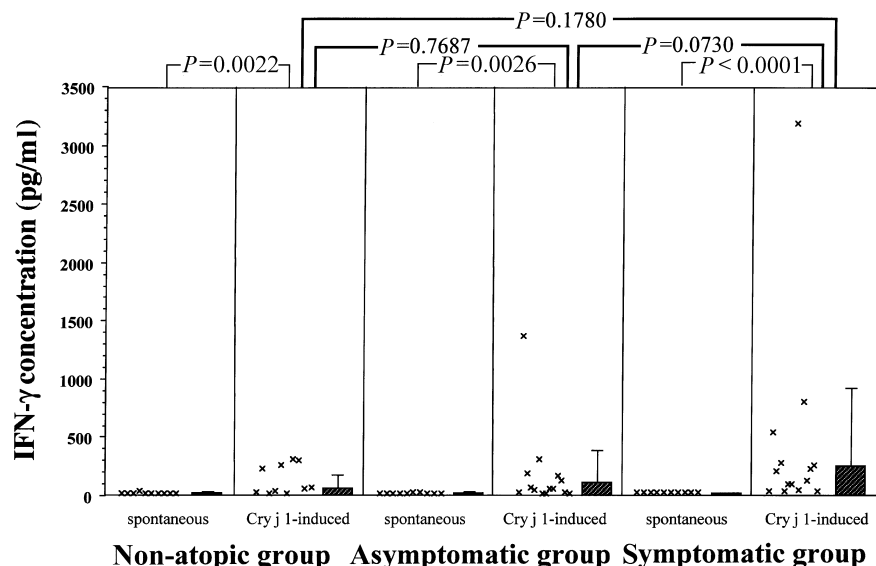


Fig. 3. The level of IFN- γ produced in the culture supernatants. The levels of IFN- γ produced by Cry j 1-stimulated PBMCs did not differ significantly among the three groups (non-atopic group versus asymptomatic group $P = 0.7687$; non-atopic group versus symptomatic group $P = 0.1780$; asymptomatic group versus symptomatic group $P = 0.0730$).

IFN- γ synthesis in each group (non-atopic group $P = 0.0002$; asymptomatic group $P = 0.0026$; symptomatic group $P < 0.0001$). The levels of IFN- γ in Cry j 1-stimulated culture supernatants did not differ significantly among the non-atopic group, the asymptomatic group and the symptomatic group (non-atopic group versus asymptomatic group $P = 0.7687$; non-atopic group versus symptomatic group $P = 0.1780$; asymptomatic group versus symptomatic group $P = 0.0730$).

DISCUSSION

During the pollen season, IL-4 stimulated PBMCs of atopic donors produced more IgE than IL-4-stimulated PBMCs of non-atopic donors whereas out of the season no difference was observed between these two groups [7]. Natural exposure to the pollen might thus affect the *in vitro* response of PBMCs to the allergen. In our study, PBMCs of each individual were therefore obtained during the cedar pollen season. Cry j 1 and Cry j 2 are known to be the major allergens of Japanese cedar pollen [8, 9]. Cry j 1 is localized on the exine outside layer, and Cry j 2 is contained in the amyloplast inside of the pollen, and pollen extracts contain Cry j 1 and Cry j 2 at an approximate ratio of 4:1. Since Cry j 1 was recently reported to be the more important allergen [10], Cry j 1 was therefore used to stimulate PBMCs in this study.

The aim of the study was to investigate whether the quantities of IgE, IL-5 and IFN- γ produced by PBMC cultures in response to the cedar pollen allergen differed between asymptomatic and symptomatic individuals with detectable levels of cedar pollen-specific IgE in serum. The levels of IgE produced by Cry j 1-stimulated PBMCs from the asymptomatic and symptomatic groups were significantly higher than those from the non-atopic group, but did not differ between the asymptomatic and symptomatic groups. Our results suggest that the sensitized individuals, irrespective of their asymptomatic or symptomatic status, have more enhanced IgE synthesis in response to the pollen allergen

than the non-atopic individuals, but that an enhanced IgE synthesis in response to the pollen allergen does not always play an important part for clinical allergic manifestation during the pollen season. To the best of our knowledge, this is the first *in vitro* study to demonstrate that allergen-induced IgE synthesis played little role in the clinical manifestation of sensitized individuals.

Although the peak of allergen-induced IgE synthesis by PBMCs usually takes 10 days or more, PBMCs were stimulated with Cry j 1 for only 4 days in our study, because our study was designed to determine simultaneously the allergen-induced synthesis of IL-5 and IFN- γ . Thus the level of IgE produced by Cry j 1-stimulated PBMCs is likely to be much lower than the peak level. However, the IgE produced by Cry j 1-stimulated PBMCs is likely to be the result of the allergen-derived synthesis, but not of the allergen-independent spontaneous synthesis, because the level of spontaneous IgE production in the 4-days allergen-free cultures was much lower. The peak level of IgE synthesis by the allergen-stimulated PBMCs might possibly differ between the asymptomatic and symptomatic groups. The present study was exclusively focused on the allergen-induced IgE synthesis, but other immunoglobulins such as IgG and IgA might be involved in the clinical manifestation in sensitized individuals. A further study focused on the above-mentioned questions is currently being considered in our laboratory.

Cytokines could be divided into two types according to their physiological properties [11]. One is produced by Th1-like cells and the other by Th2-like cells. Different lines of evidence for the importance of Th2-type cytokines in the pathogenesis of allergic inflammation have emerged [1]. Interleukin-5 is one of the most important cytokines involved in allergic diseases, which is secreted by activated Th2-like cells and is particularly implicated in the pathogenesis of allergic inflammatory events in the airway, because it selectively promotes the differentiation, proliferation, survival and effector function of eosinophils [12–14].

Expression of mRNA for IL-5 has been detected in bronchial biopsies of asthmatics, and the expression of IL-5 mRNA has tended to increase in severe disease [15]. Treatment with anti-IL-5 MoAbs could inhibit the development of eosinophilia and hyperresponsiveness [16]. This cumulative evidence indicates that IL-5 is closely involved in airway eosinophilia and in the development of hyperreactivity.

Because eosinophilic inflammation is an important component of symptomatic episodes of seasonal allergic rhinitis, we have investigated whether the levels of IL-5 produced by Cry j 1-stimulated PBMCs differ between asymptomatic and symptomatic individuals. Although we did not determine the spontaneous and allergen-induced synthesis of IL-5 in each individual, IL-5 above the sensitivity was not produced by PBMCs in the absence of Cry j 1. Therefore IL-5 produced by PBMCs in the presence of Cry j 1 was likely to be the result of IL-5 synthesis in response to the cedar pollen allergen. In our study, the levels of IL-5 produced by Cry j 1-stimulated PBMCs did not differ significantly between the non-atopic group and the asymptomatic group, which was not likely to suggest that IL-5 played a central part in sensitization to the pollen.

The levels of IL-5 produced by Cry j 1-stimulated PBMCs were significantly higher in the symptomatic group than in the asymptomatic group, as well as in the non-atopic group, which might suggest that IL-5 played an important part in the clinical manifestation of seasonal allergic rhinitis in patients sensitized to the pollen. Our study has thus demonstrated that IL-5 significantly contributes to the symptomatic episode of seasonal allergic rhinitis in sensitized individuals. Levels of IL-5 in bronchoalveolar lavage fluid are reported to be significantly higher in patients with symptomatic asthma compared with those with asymptomatic asthma [17]. In addition, an enhanced IL-5 production by Th cells is observed in both atopic and non-atopic asthmatics [18]. Our findings are in agreement with these two reports [17, 18] in that IL-5 holds the key to clinical manifestation of allergic disorders.

Not only Th2 response but also Th1 response is considered to differ between atopic and non-atopic individuals. Interferon- γ secreted by Th1-like cells is generally considered to play reciprocal activities of Th2-type cytokines, because IFN- γ inhibits the IgE synthesis by B lymphocytes and the development of Th2 clones [19]. Indeed, stimulated peripheral blood T lymphocytes from atopic individuals are reported to secrete significantly less IFN- γ than those from normal controls [20]. We thus compared the production of IFN- γ by Cry j 1-stimulated PBMCs in the non-atopic group, the asymptomatic group, and the symptomatic group. The levels of IFN- γ synthesis by Cry j 1-stimulated PBMCs did not differ significantly among these three groups. Our data are not therefore likely to suggest that Th1 response to the pollen allergen plays an important role in the sensitization as well as symptomatic episodes of seasonal allergic rhinitis, which is in line with our previous serological study of seasonal allergic rhinitis [6].

In conclusion, our study has demonstrated that Japanese cedar pollen-induced synthesis of IL-5, but not of IgE or IFN- γ , is

likely to be a key important mechanism linked to the symptomatic episode of seasonal allergic rhinitis in individuals sensitized to the pollen. Additionally, our study suggests that medical control of IL-5 synthesis in response to the pollen may provide a clue to possible prevention of future allergic symptoms in individuals sensitized to the pollen. However, it is necessary, for a definite understanding of the clinical role of Th response and IgE response in the clinical manifestation of sensitized individuals, to investigate the response of PBMCs to non-specific as well as allergen-specific stimulation during as well as outside of the pollen season.

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