

Japanese cedar pollen-specific interleukin-4 production develops immediately after the first exposure to pollens in infants with atopic dermatitis

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Summary

Background Japanese cedar pollens (JCPs) spread over most areas of Japan from February to April and cause pollenosis. While IgE synthesis against JCPs starts after age 1, it remains to be clarified when JCP-specific T helper cells acquire the ability to produce IL-4, a cytokine that induces IgE synthesis.

Objective This study aimed to clarify when the sensitization of T cells to JCPs develops.

Methods Peripheral blood mononuclear cells from 153 children with atopic dermatitis (AD) aged 0–15 years were stimulated with a standardized JCP allergen. As parameters of T cell responsiveness, lymphocyte proliferation and the production of IL-4 and IFN- γ were measured.

Results T cell responses against JCPs were negative before March in infants with AD who had never been exposed to JCPs or who were exposed for less than a month (nine, seven and nine subjects for lymphocyte proliferation, cytokine production and IgE synthesis, respectively). Lymphocyte proliferation distinctly increased in 67.6% (23/34) of infants with AD examined between March and June. JCP-specific IL-4 production was observed in 56.0% (14/25) of infants with AD examined between March and June. Correspondingly, a slight increase in the level of serum JCP-specific IgE antibody was seen in 17.2% (five of 29) of infants with AD examined between April and June.

Conclusion These results demonstrate that the sensitization of T cells to JCPs is effectively completed within a few months after the first exposure to JCPs.

Keywords atopic dermatitis, IFN- γ , IgE antibody, IL-4, infants, Japanese cedar pollen, lymphocyte proliferation

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Introduction

While atopic dermatitis (AD) frequently develops during infancy, pollenosis is rare in younger children [1, 2]. Correspondingly, IgE antibody specific to pollens is negative in those subjects [2, 3]. However, several investigators reported that the responses of T cells to pollens rose in infants, neonates and fetuses [4–7]. This suggests that the sensitization of T cells to pollens develops much earlier than IgE synthesis. However, the relation between the time of exposure to pollens and the development of T cell responsiveness has not yet been determined.

In this study, we examined T cell responsiveness to Japanese cedar pollens (JCPs), a primary cause of pollenosis in Japan [8, 9], in children with AD. One special purpose of this study was to clarify the gap between the time of contact with JCPs and the acquisition of T cell responsiveness to JCPs in infants. The results demonstrate that the capacity of JCP-specific T helper (Th) cells to secrete IL-4, a primary cytokine

to induce IgE synthesis, develops in infants with AD within a few months after their first contact with JCPs.

Materials and methods

Subjects

Heparinized peripheral blood was obtained at the time of routine blood examination from 153 children with AD who visited us between January 2000 and June 2003, after informed consent was obtained. AD was diagnosed using the criteria set by Hanifin and Rajka [10]. No subjects were given systemic corticosteroids. As controls, heparinized blood was similarly collected from 24 non-atopic children who did not have allergic disorders or any other serious diseases. This study was approved by the institutional ethical review board.

In the analysis of the changes in T cell responses according to the month of examination, subjects were limited to infants with AD born between May and January and examined between October and June (Fig. 1). Forty-three of 56 infants with AD met this criterion. Subjects examined between October and January were thought to be never exposed to JCPs, while those examined between February and June were regarded as being exposed to JCPs.

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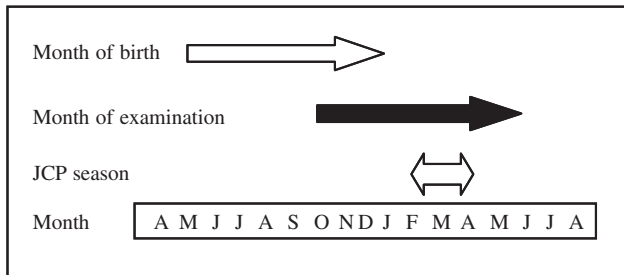


Fig. 1. Infants with atopic dermatitis (AD) enrolled in the study of the influence of the month of examination. The month of birth and the month of examination of infants with AD involved in the analyses of the relationship between the responsiveness of T cells to Japanese cedar pollens (JCPs), which spread from February to April, and the month of examination is shown.

Cell culture

Cell culture was performed according to a previously described method with some modifications [11]. Peripheral blood mononuclear cells (PBMCs) were resuspended at a concentration of 1×10^6 cells/mL in RPMI1640 medium (Sigma-Aldrich Fine Chemical, Tokyo, Japan) supplemented with 10% heat-inactivated human AB serum, 2 mM glutamine and antibiotics (100 U/mL penicillin and 100 µg/mL of streptomycin). The cell suspension was dispensed in duplicate to each well (0.5 mL/well) on a 48-well culture plate (Falcon 3078, Becton Dickinson & Co, NJ, USA). Cells were cultured for 6 days in a humidified atmosphere of 5% CO₂ at 37 °C with or without 100 Japanese Allergy Unit (JAU)/mL of standardized JCP allergen provided by the Japanese Society of Allergology (Tokyo, Japan). Ten thousand JAU per millilitre of JCP allergen contains 12.5 µg/mL of Cry j1. In some experiments, PBMCs were stimulated with house dust mite (HDM)-crude extract (50 µg/mL) (Torii Pharmaceutical Co. Ltd, Tokyo, Japan) [12] for 6 days. The concentrations of allergens and the duration of culture were determined by preliminary experiments.

The concentration of endotoxin in the medium containing 100 JAU/mL JCP allergen is less than 0.2 ng/mL. PBMCs neither proliferated nor produced IL-4 or IFN-γ by stimulation with 0.2 ng/mL lipopolysaccharide for 6 days (data not shown).

After incubation, the supernatant was collected and stored at -70 °C for the measurement of cytokines. The cells were washed twice with PBS and fixed with 70% ethanol for the measurement of lymphocyte proliferation.

Measurement of allergen-specific lymphocyte proliferation

Allergen-specific lymphocyte proliferation was measured by the use of flow cytometry, as described previously [11]. Briefly, fixed cells were washed twice with PBS, and incubated in 500 µL PBS containing 100 µg/mL RNase (Sigma) at 37 °C for 1 h. Cellular DNA was stained by 40 µg/mL propidium iodide (Sigma). The amount of DNA per cell was analysed by flow cytometry (EPICS Elite, Beckman-Coulter KK, Tokyo, Japan) and the S-phase cells that appeared in a flat region between the peaks of the G0/G1- and G2/M-phase cells were measured. The percentage of the S-phase cells was converted into a stimulation index using the following equation and

expressed as:

$$\text{SIF} = \frac{\text{S-phase cells (\%)} \text{ in a stimulated sample}}{\text{S-phase cells (\%)} \text{ in an unstimulated sample}} \times 100$$

where SIF is the stimulation index measured by flow cytometry.

Measurement of cytokines and IgE antibody specific to JCPs

IL-4 and IFN-γ in the culture supernatant were measured with the ELISA kit (Cytoscreen human IL-4 US (sensitivity, 200 fg/mL) and IFN-γ (sensitivity, 1.0 pg/mL), BioSource International, Camarillo, CA, USA). The difference between the amount of cytokine in a stimulated and unstimulated sample was regarded as JCP-specific cytokine production in each subject.

JCP-specific IgE antibody (JCP-IgE) was measured using a CAP-RAST RIA kit (Pharmacia KK, Tokyo, Japan).

Statistics

Statistical data are presented as the median and range. The significance of differences was estimated using Mann-Whitney's *U*-test. Results were regarded as significant if the *P*-value was lower than 0.05.

Results

Serum IgE levels against JCPs

The level of serum JCP-IgE was very low in infants with AD, and then rapidly rose with age after age 1 (Fig. 2). However, serum JCP-IgE was positive (>0.35 UA/mL) in a small number of infants with AD (Fig. 2), although the level was low (median, 0.98 (range, 0.55–1.29) UA/mL, *n* = 6). Infants with positive JCP-IgE were limited to those examined in and after April (Fig. 3).

Lymphocyte proliferation against JCPs

In contrast to JCP-IgE, JCP-specific lymphocyte proliferation was already high in infants with AD (SIF, 223 (73–1070), *n* = 56) and comparable with that in older children (Fig. 4). While JCP-specific lymphocyte proliferation was completely negative in nine infants with AD examined up until February, it was positive in 67.6% (23/34) of AD infants examined in

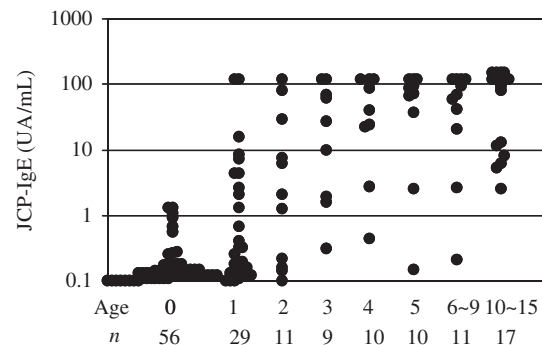


Fig. 2. Age-dependent changes in serum Japanese cedar pollen (JCP)-IgE levels in children with AD. The level of serum JCP-IgE in children with AD is shown according to age (years). *n*, the number of subjects.

and after March (Fig. 5a). The rise of lymphocyte proliferation against JCPs occurred a month earlier than that of the serum JCP-IgE level.

Although HDM-specific lymphocyte proliferation also increased in infants with AD, it did not show a clear alteration according to the month of examination (Fig. 5b).

In control subjects, JCP-specific lymphocyte proliferation examined between March and June was not significantly different from that examined between October and February either in infants (0 year) or in older children (1–15 years) (data not shown).

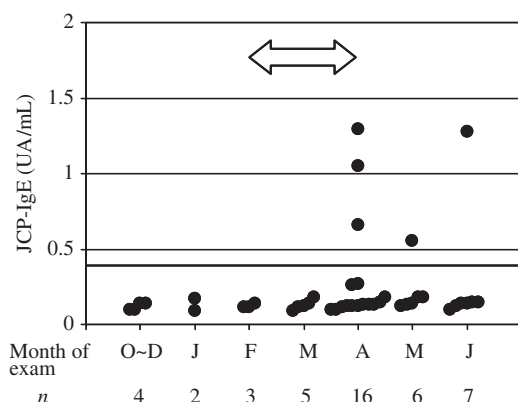


Fig. 3. Changes in serum Japanese cedar pollen (JCP)-IgE levels in infants with atopic dermatitis (AD). The level of serum JCP-IgE in infants with AD is shown according to the month of examination. An arrow in the figure indicates the season of JCPs. A horizontal line indicates the upper limit of the normal range of JCP-IgE (0.35 UA/mL). *n*, the number of subjects.

IL-4 production against JCPs

IL-4 was produced by stimulation of PBMCs with JCPs in many children with AD (Fig. 6a). In infants with AD, JCP-specific IL-4 production was seen only in those examined in and after March (56.0% (14/25)) but not in seven subjects examined between October and February (Fig. 7a). In older children with AD, JCP-specific IL-4 production examined between March and June was higher than but not significantly different from that examined between October and February (910 (<200–6850) fg/mL, *n* = 7, vs. 280 (<200–2025) fg/mL, *n* = 5). The production of IL-4 in infants with AD (280 (<200–8350) fg/mL, *n* = 25) was not significantly different from that in older children with AD (910 (<200–11 760) fg/mL, *n* = 13) (Fig. 6a).

In control subjects, there were no significant differences in JCP-specific IL-4 production between infants (0 year) and older children (1–15 years) (<200 (<200–215) fg/mL, *n* = 9, vs. <200 (<200) fg/mL, *n* = 8). IL-4 production in control subjects examined between March and June was not significantly different from that examined between October and February (<200 (<200–200) fg/mL, *n* = 8, vs. <200 (<200) fg/mL, *n* = 8).

IFN- γ production against JCPs

JCPs also induced IFN- γ production in infants with AD (Fig. 6b). IFN- γ production was negative in seven subjects examined up until February and became positive thereafter in 13 of 24 subjects (54.2%) (Fig. 7b). In older children with AD, JCP-specific IFN- γ production examined between March and June was higher than but not significantly different from that

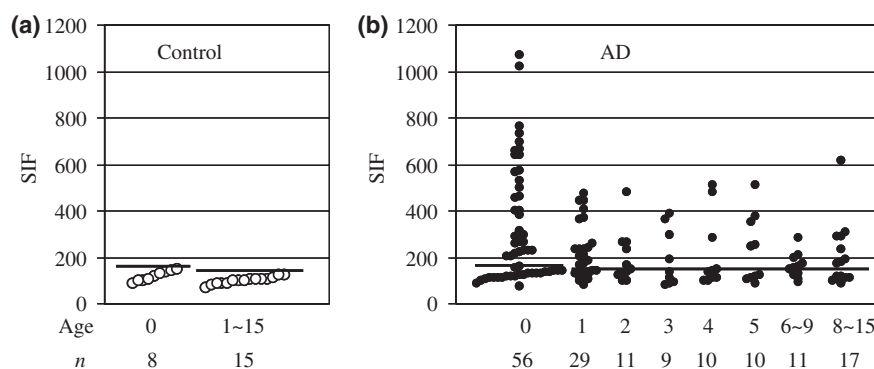


Fig. 4. Japanese cedar pollen (JCP)-specific lymphocyte proliferation in children with atopic dermatitis (AD). Lymphocyte proliferation upon stimulation with JCPs in control subjects (a) and in children with AD (b) is shown according to age (years). The result is expressed as stimulation index measured by flow cytometry (SIF). Horizontal lines in the figures indicate the upper limit of the normal range of JCP-specific SIF (150 for infants and 130 for older children). *n*, the number of subjects.

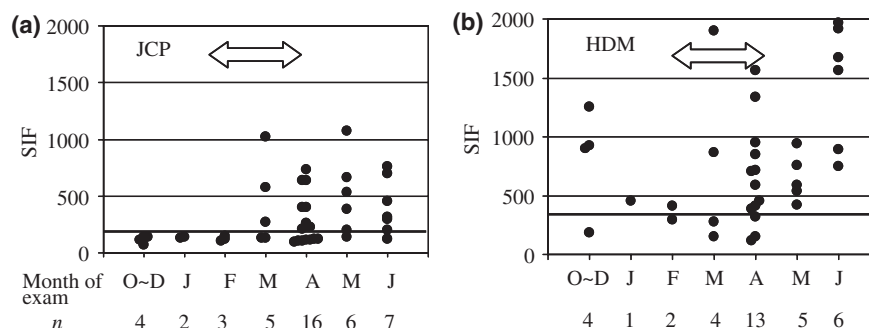


Fig. 5. Changes in Japanese cedar pollen (JCP)-specific lymphocyte proliferation with the month of examination. JCP-specific lymphocyte proliferation in infants with AD is shown according to the month of examination (a). The result is expressed as SIF. For comparison, HDM-specific lymphocyte proliferation is shown in parallel (b). Arrows in the figures indicate the season of JCPs. Horizontal lines indicate the upper limits of the normal range for JCPs (150) and HDM (300). *n*, the number of subjects.

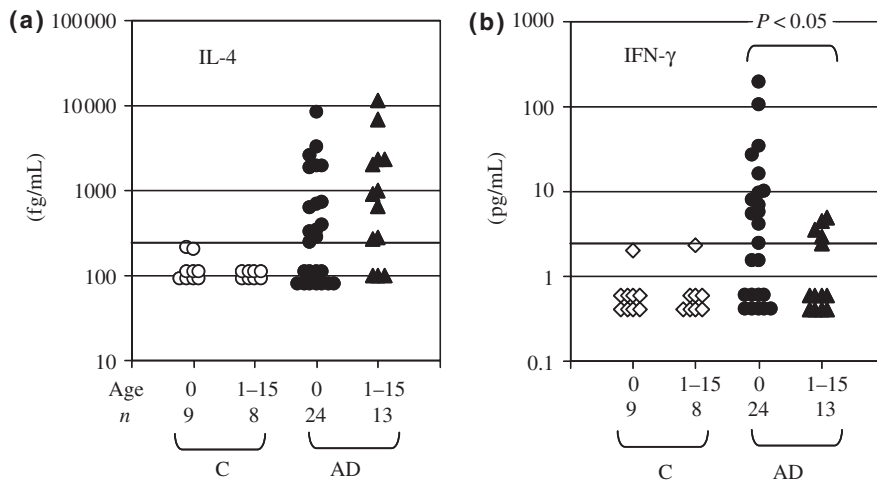


Fig. 6. IL-4 and IFN- γ production upon stimulation with Japanese cedar pollens (JCPs) in children with atopic dermatitis (AD). The production of IL-4 (a) and IFN- γ (b) by peripheral blood mononuclear cells upon stimulation with JCP allergen is shown in children with AD (0 and 1–15 years) and in control subjects (0 and 1–15 years). Infants with AD were limited to those examined between March and June. Horizontal lines indicate the upper limits of the normal range for IL-4 (250 fg/mL) and IFN- γ (2.5 pg/mL). C, control subjects. *n*, the number of subjects.

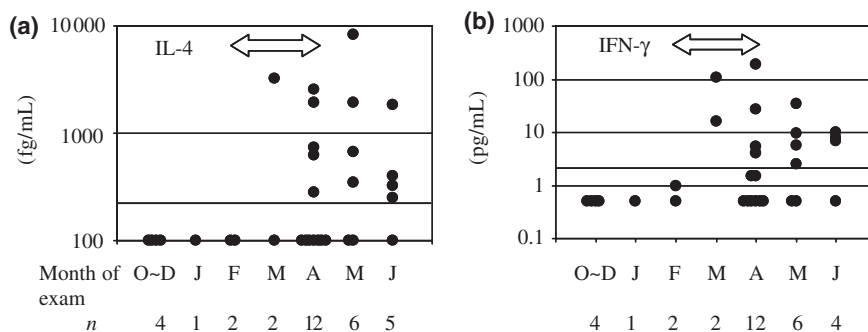


Fig. 7. Changes in IL-4 and IFN- γ production with the month of examination. The production of Japanese cedar pollen (JCP)-specific IL-4 (a) and IFN- γ (b) in infants with atopic dermatitis (AD) is shown according to the month of examination. An arrow in the figure indicates the season of JCP. Horizontal lines indicate the upper limits of the normal range for IL-4 (250 fg/mL) and IFN- γ (2.5 pg/mL). *n*, the number of subjects.

examined between October and February (2.4 (<1 –4.9) pg/mL, $n = 7$, vs. <1.0 (<1.0 –3.0) pg/mL, $n = 5$). The production of IFN- γ in infants with AD examined between March and June (3.3 (<1.0 –191) pg/mL, $n = 24$) was significantly higher than that in older children with AD (<1.0 (<1.0 –4.9) pg/mL, $n = 13$, $P < 0.05$) (Fig. 6b), although the difference was not significant if older children with AD were limited to those examined between March and June.

In control subjects, there were no significant differences in JCP-specific IFN- γ production between infants (0 year) and older children (1–15 years) (<1.0 (<1.0 –2.0) pg/mL, $n = 9$, vs. <1.0 (<1.0 –2.3) pg/mL, $n = 8$). IFN- γ production in control subjects examined between March and June was not significantly different from that examined between October and February (<1.0 (<1.0 –2.3) pg/mL, $n = 8$, vs. <1.0 (<1.0 –2.0) pg/mL, $n = 8$).

Discussion

JCP is a major tree pollen allergen that causes pollenosis in Japan [8, 9]. Pollenosis is thought to be caused by an immediate hypersensitivity reaction in humans. Indeed, JCP-IgE is usually positive in patients with pollenosis against JCPs. JCP-IgE rises after age 1, and is practically negative in infants (Fig. 2). Consistently, typical symptoms of pollenosis are not seen in infants. However, in this study, we found that the development of sensitization of T cells to JCPs occurs in

infants. In contrast to JCP-IgE, lymphocyte proliferation and cytokine production against JCPs increased in many infants with AD examined between March and June. The levels of these JCP-specific T cell responses in those infants were comparable with those in older children with AD.

IL-4 production against HDM also increased in infants with AD previously to IgE synthesis [13, 14]. However, the gap between the time of the first contact with allergens and the initiation of IL-4 production is still unclear. In this study, we demonstrated that the gap for JCPs is not longer than a few months. Thus, the sensitization of T cells to JCPs is practically completed in many infants within the first year of life. This implies the necessity of countermeasures to avoid contact with pollens during the first season in life since it might seriously influence IgE synthesis in the future as discussed below.

Many investigators found that the incidence of pollenosis is high in individuals born in autumn or winter and low in those born in spring [15–21]. Although the cause of this phenomenon has not yet been clarified, the exposure to pollens before 6 months old is regarded as playing a critical role [20, 21]. On the other hand, it is also noted that the incidence of AD is higher in infants born in autumn and winter in western countries as well as in Japan [22–25]. Since AD usually develops before 6 months of age [10], most infants with AD born in autumn and winter already show symptoms at the beginning of the first season of tree pollens such as JCPs or birch pollens. Because AD damages the barrier function of the skin,

soluble pollen allergens may penetrate the affected skin and stimulate the local immune system, such as Langerhans cells and T cells.

In this study, we demonstrated that the sensitization of T cells against JCPs is effectively completed in many infants with AD during or immediately after the first JCP season. AD in infants appears to act as a facilitating factor for the sensitization of T cells to JCPs. Thus, an increased risk of AD in infants born in autumn and winter is suggested to have a close relationship with a rise in the incidence of pollenosis in subjects born in the same period. The fact that symptoms of AD in infants usually subside within a few years after birth, while those of pollenosis usually develop thereafter, may hinder people from perceiving the relation between the two.

Intrauterine sensitization to pollen allergens is suggested [4–7]. This study demonstrated that T cell responses against JCPs were negative or very weak in control subjects or infants with AD never exposed to JCPs compared with infants with AD exposed to JCPs. This suggests that the influence of postnatal contact with JCPs on the human immune system is much stronger than that of prenatal contact. However, in this study, the number of subjects without postnatal contact with JCPs may not be enough to draw a conclusion. More studies about T cell responses against JCPs in a large number of infants with predisposition to pollenosis, for example those infants with positive family history of pollenosis, by the same method is necessary to precisely estimate the influence of prenatal contact with pollen allergens.

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