

Japanese Cedar-Pollen-Specific IL-5 Production in Infants with Atopic Dermatitis

Mitsuaki Kimura Makiko Obi Megumu Saito

Department of Allergy and Clinical Immunology, Shizuoka Children's Hospital, Shizuoka, Japan

Key Words

Atopic dermatitis · IL-5 · Infants · Japanese cedar pollen · Pollenosis · Season

Abstract

Background: Although food allergens and house dust mites are thought to contribute to the development of atopic dermatitis (AD) in infants, the influence of pollens has not yet been well defined. **Objective:** This study attempted to clarify the effect of Japanese cedar pollens (JCPs), which are scattered in Japan between February and April, on AD in infants. **Methods:** In the first study, seasonal changes in the number of patients who first visited our institute due to AD were analyzed by reviewing medical records of 184 infants with AD. In the second study, peripheral blood mononuclear cells from 81 children (50 infants <1 year and 31 older children) with AD were stimulated with a standardized JCP allergen. The production of IL-5 was measured and its correlation with the severity of AD in infants was analyzed. **Results:** The number of infants who first visited the institute due to AD peaked in spring. JCP-specific IL-5 production began to increase immediately after the first contact with JCPs in infants with AD. The production of IL-5 in infants with AD exposed to JCPs was significantly higher than in control subjects ($p < 0.002$) or in AD infants never exposed to JCPs ($p < 0.02$). There was a significant positive correlation between IL-5 production and the severity of AD

in infants ($p < 0.0001$). **Conclusion:** These results suggest that JCPs play an important role in the aggravation of infantile AD in spring by inducing IL-5 production.

Copyright © 2004 S. Karger AG, Basel

Introduction

Food allergens have been ascribed a critical role in the development of atopic dermatitis (AD) in infants [1]. We showed that house dust mite (HDM) also makes a significant contribution to the development of AD in infants by inducing IL-5 production, a proinflammatory Th2 cytokine, by HDM-specific Th2 cells [2]. However, it still remains unclear whether pollens influence the clinical course of AD in infants.

Japanese cedar pollens (JCPs), which spread from February to April, represent seasonal pollen allergens in Japan [3, 4]. Because serum IgE antibody against JCPs is usually negative in infants with AD [5], JCPs has never been regarded as a cause of infantile AD. However, we recently demonstrated that JCP-specific IL-4 and IFN- γ production by peripheral blood mononuclear cells (PBMCs) rose immediately after the first contact with JCPs in infants with AD [5]. Thus, JCPs may also affect the symptoms of AD in infants by inducing proinflammatory cytokines such as IL-5.

In this study, we demonstrated that JCP-specific IL-5 production by PBMCs actually increased in infants with

Table 1. Subjects

Study	Subjects	Remarks	
1st study	184 infants with AD (<1 year)	Born between January 1, 1995, and December 31, 1997	
2nd study	81 children with AD (0–15 years)	50 infants (<1 year)	6 (2–11) months ^a
		31 older children (>1 year)	3 (1–15) years ^a
	22 control subjects (0–12 years)	10 infants (<1 year)	5 (2–10) months ^a
		12 older children (>1 year)	3 (1–12) years ^a
^a Median and range.			

AD immediately after the first contact with JCPs. Moreover, a significant positive correlation was seen between IL-5 production and the severity of AD symptoms. We also found that the number of infants with AD who visited physicians sharply increased during spring. Thus, JCPs appear to contribute to the aggravation of infantile AD in spring.

Subjects and Methods

Subjects

One hundred and eighty-four infants (<1 year) with AD who were born between January 1, 1995, and December 31, 1997, and first referred to us before the age of 1 year were enrolled in the first study which aimed to estimate the seasonal influence on the incidence of infantile AD (table 1). The month of the first visit to our institute was extracted from the medical records and regarded as the time of aggravation of AD symptoms.

Eighty-one children (50 infants and 31 older children) with AD who visited us between January 1, 2000, and February 2004 were included (after informed consent was obtained) in the second study which tried to characterize JCP-specific IL-5 production in atopic infants (table 1). IgE antibody against egg and/or cow's milk was positive in 41 of 50 (82%) infants with AD. Infants born and examined from the previous May to January were regarded as infants with no JCP exposure. No subjects were given systemic corticosteroids. As controls, blood samples were similarly collected from 22 children without either allergic disorders such as AD, bronchial asthma, food allergy and pollenosis or any other serious diseases.

AD was diagnosed using the criteria set by Hanifin and Rajka [6]. The severity of AD was determined according to the criteria of the Japanese Dermatological Association into mild (no severe skin lesions), moderate (severe skin lesions on less than 10% of the body surface), severe (severe skin lesions on 10–30% of the body surface), and very severe (severe skin lesions on more than 30% of the body surface).

Cell Culture

Cell cultures were performed according to a previously described method with some modifications [2]. Heparinized peripheral

blood was obtained from subjects at routine blood examination. PBMCs were obtained by centrifugation of heparinized peripheral blood over Lymphocyte Separation Medium (ICN Biomedicals, Aurora, Ohio, USA) and resuspended at a concentration of 1×10^6 cells/ml in RPMI 1640 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 into each well (0.5 ml/well) on a 48-well culture plate (Falcon 3078, Becton Dickinson, Franklin Lakes, N.J., USA). Cells were cultured for 6 days in a humidified atmosphere of 5% CO₂ at 37°C with 100 Japanese Allergy Units (JAU)/ml standardized JCP allergen provided by the Japanese Society of Allergology. After incubation, the supernatant was stored at –70°C for the measurement of IL-5.

Ten thousand JAU/ml JCP allergen contain 12.5 µg/ml of Cry j 1. The largest IL-5 production is seen by stimulation of PBMCs with 50–100 JAU/ml standardized JCP allergen, which is larger than that obtained by stimulation with 5 µg/ml purified Cri j 1 protein (Hayashibara Biochemicals Laboratories, Okayama, Japan; data not shown). Although a small amount of IL-5 production is observed after a 2-day culture of PBMCs with JCP allergen, it increases with the length of the culture period up to 6 days (data not shown).

The concentration of endotoxin in the medium containing 100 JAU/ml JCP allergen is less than 0.4 ng/ml. LPS at a concentration of up to 1 ng/ml does not induce any IL-5 production by PBMCs after 6 days of culture (data not shown).

Measurement of IL-5

IL-5 in the culture supernatant was measured in duplicate by the use of an enzyme-linked immunosorbent assay kit with a sensitivity of 1.0 pg/ml (Cytoscreen human IL-5, BioSource International, Camarillo, Calif., USA). The difference between the amount of cytokine in a stimulated and unstimulated sample was shown as JCP-specific IL-5 production.

Statistics

Data on IL-5 production were expressed as the median and range. The significance of the differences and correlations were estimated using the Mann-Whitney U test and Spearman's rank correlation coefficient, respectively. Results were regarded as significant if the p value was lower than 0.05.

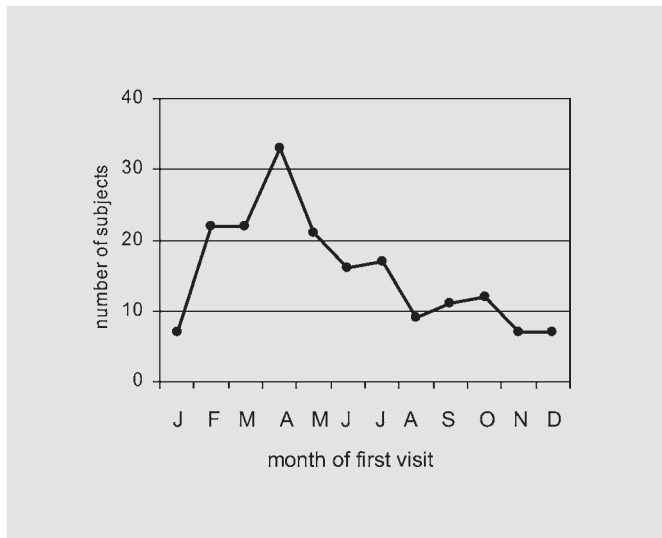


Fig. 1. Seasonal changes in the number of infants with AD who first visited our institute during the indicated months due to symptom aggravation are given. Only the first letters of the months are given.

Results

Seasonal Changes in the Incidence of Infantile AD

We first estimated the seasonal influence on the symptoms of infantile AD by analyzing the changes in the number of infants who were first referred to us because of AD aggravation. There was a marked seasonal alteration in the number of AD infants who first visited our institute: namely, the number sharply increased from February, peaked in April and then rapidly declined to the lowest level in August (fig. 1), but this alteration was less obvious in older children (data not shown).

IL-5 Production following JCP Stimulation

The results of the first study suggest the presence of some aggravating factors of infantile AD in spring. JCP, a representative pollen allergen in Japan in spring, was chosen as a candidate for such factors, and JCP-specific IL-5 production by PBMCs was characterized in infants with AD. Upon stimulation with JCPs, IL-5 production by PBMCs was significantly higher in infants with AD exposed to JCPs than in control subjects ($p < 0.002$) or in infants with AD never exposed to JCPs ($p < 0.02$; fig. 2).

Among AD patients exposed to JCPs, JCP-specific IL-5 production was even observed in 2- to 4-month-old infants (table 2). It peaked in infants aged 5–11 months and then decreased with age.

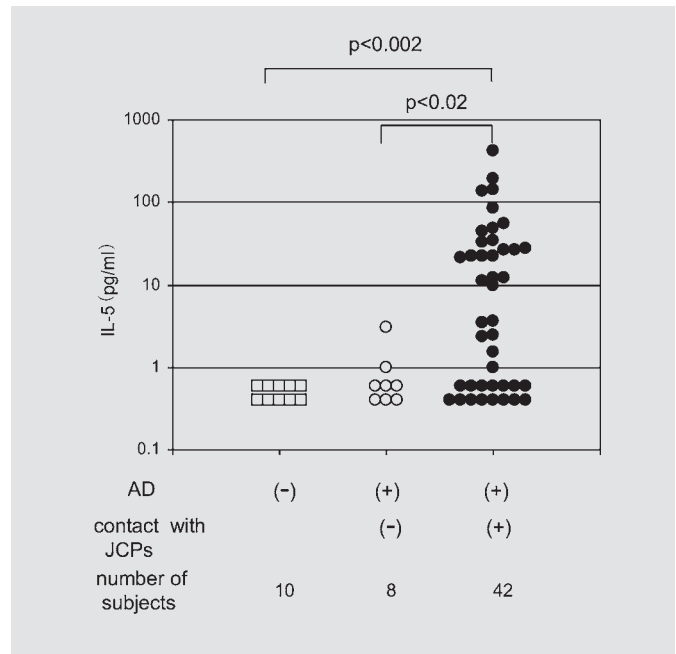


Fig. 2. JCP-specific IL-5 production in infants with AD is shown in comparison with control subjects. □ = Control subjects; ● = infants with AD having postnatal JCP contact; ○ = infants with AD who were born and examined from the previous May to January with no known JCP exposure.

Table 2. Changes in IL-5 production with age

Age	Subjects	Median IL-5 pg/ml
<i>AD</i>		
2–4 months	9 ^a	1.5 (<1–195)
5–7 months	20 ^a	6.8 (<1–419)
8–11 months	13 ^a	12.0 (<1–136)
1 year	14	3.4 (<1–89)
2–15 years	17	2.0 (<1–610)
<i>Controls</i>		
2–10 months	10 ^b	<1.0 ^c
1–12 years	12	<1.0 ^c

Ranges are given in parentheses.

^a Infants who were born and examined from the previous May to January were regarded as never being exposed to JCPs and excluded from the analysis.

^b 4 infants were exposed to JCPs.

^c IL-5 production was less than 1 pg/ml in all subjects.

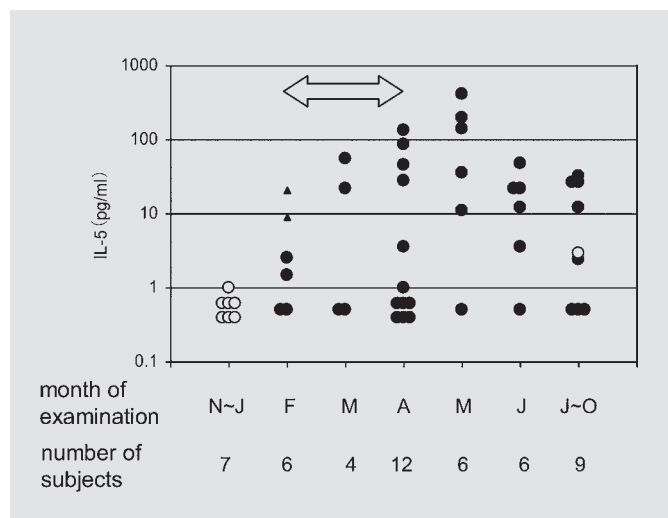


Fig. 3. IL-5 production increased immediately after exposure to JCPs. The alteration in the JCP-specific IL-5 production according to the month of examination is shown. The arrow in the figure indicates the JCP season. ○ = Infants born and examined from the previous May to January and never exposed to JCPs; ● = infants once exposed to JCPs; ▲ = infants born in the previous March and twice exposed to JCPs. Capital letters indicate the respective months.

Changes in IL-5 Production according to the Month of Examination

Marked changes in JCP-specific IL-5 production were seen according to the month of examination (fig. 3A). IL-5 production began to increase from February, when the JCP season started, and formed a conspicuous peak in May. Thereafter, it showed a sharp decline. However, the month of examination had a negligible effect on alterations in IL-5 production by PBMCs following HDM stimulation (data not shown).

Correlation between JCP-Specific IL-5 Production and the Severity of Infantile AD

There was a significant positive correlation between JCP-specific IL-5 production and the severity of AD in infants ($p < 0.0001$; fig. 4).

Discussion

In Japan, the number of infants with AD paying a first visit to the physician due to symptom aggravation distinctly increases during spring (fig. 1). Although Aoki et al. [7] reported similar results, they did not refer to JCPs,

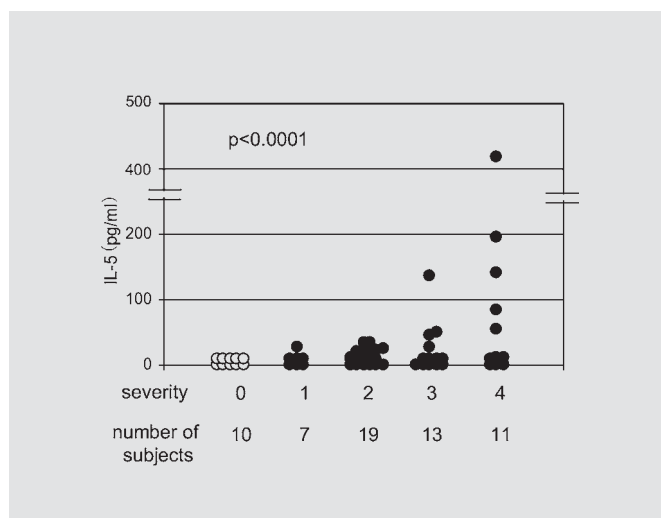


Fig. 4. Correlation of JCP-specific IL-5 production with AD severity in infants. ○ = Control subjects; ● = infants with AD; 0 = no symptoms; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe.

a major tree allergen in Japan in spring, as a possible cause of AD. In this study, we attempted to shed light on the role of JCPs in the aggravation of infantile AD in spring.

Sensitization to pollen allergens is thought to take more than a year because IgE antibody against pollens is not produced before 1 year of age [5, 8, 9]. Hence possible relationships between JCPs and the aggravation of infantile AD during the first spring after birth have not been considered. However, we recently demonstrated that PBMCs from infants with AD produced a large amount of IL-4 upon stimulation with JCPs immediately after the first exposure to JCPs [5], indicating that JCP-specific Th2 cells develop in infants with AD prior to the beginning of IgE synthesis.

IL-5 is a representative Th2 cytokine and plays an important role in the pathogenesis of AD [10]. If JCPs similarly induce IL-5 production by PBMCs in infants with AD who have not yet produced JCP-specific IgE, it could contribute to the deterioration in infantile AD in spring. As was shown in this study, JCPs can actually induce increased IL-5 production in infants with AD. Moreover, there is a significant positive correlation between JCP-specific IL-5 production and the severity of AD in infants. Thus, JCPs seem to play a significant role in the aggravation of infantile AD in spring. Although infants are presently not protected from pollens, countermeasures against pollens may be recommended in in-

infants with AD, especially in those with severe AD, in order to prevent the aggravation of symptoms.

It remains to be elucidated why JCP-specific IL-5 production peaked in May, when airborne JCPs are no longer present. Possibly, Japanese cypress pollens which spread in April and May (after JCPs) may be responsible for this phenomenon because they share common antigenicity with JCPs [11]. On the other hand, JCPs have recently been demonstrated in house dust [12] and may remain for several months after the JCP season in houses of patients with lingering symptoms of pollenosis due to JCPs [Enomoto, pers. commun.]. Thus, indoor JCPs may also have relevance to this phenomenon.

The production of JCP-specific IL-5 in children with AD peaked during the first year of life (table 2). This is quite different from the changes in the serum level of JCP-specific IgE antibody with age since JCP-specific IgE

antibody is insignificant in infants but rises after 1 year of age [5]. Presently, IgE antibody is almost the only routinely available laboratory parameter apart from eosinophils indicating the cause and mechanism of allergic disorders. Consequently, it seriously influences the knowledge on the pathological significance of a given allergen. This study stresses the importance of the examination of allergen-specific cytokine production for a correct understanding of the significance of JCPs in the development of allergic disorders including AD in infants.

Some investigators have suggested intrauterine sensitization of fetuses to pollens [13–16]. However, JCP-specific IL-5 production in infants with AD without any postnatal exposure to JCPs was distinctly lower compared to those with postnatal JCP exposure (fig. 2), suggesting that the influence of postnatal exposure to JCPs predominates over the prenatal contact.

References

- 1 Sly RM: Atopic dermatitis; in Behrman RE, Kliegman RM, Jenson HB (eds): Nelson Textbook of Pediatrics, ed 16. Philadelphia, Saunders, 2000, pp 680–684.
- 2 Kimura M, Tsuruta S, Yoshida T: Correlation of house dust mite-specific lymphocyte proliferation with interleukin 5 production, eosinophilia, and the severity of symptoms in infants with atopic dermatitis. *J Allergy Clin Immunol* 1998;101:84–89.
- 3 Okuda M, Shida T: Clinical aspects of Japanese cedar pollenosis. *Allergol Int* 1998;47:1–8.
- 4 Ozasa K, Dejima K, Takenaka H: Prevalence of Japanese cedar pollenosis among schoolchildren in Japan. *Int Arch Allergy Immunol* 2002;128:165–167.
- 5 Kimura M, Obi M, Saito M: Japanese cedar pollen-specific interleukin-4 production develops immediately after the first exposure to pollens in infants with atopic dermatitis. *Clin Exp Allergy* 2004;34:1032–1036.
- 6 Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl* (Stockh) 1980;92:44–47.
- 7 Aoki T, Kojima M, Adachi J, Fukuzumi T, Endo K, Sumitsuji H, Kobayashi E: Seasonal variation in the month of first visit for atopic dermatitis patients. *Allergol Int* 1998;47:137–142.
- 8 Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB: Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child* 1985;60:727–735.
- 9 Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U: Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999;103:1173–1179.
- 10 Leung DYM: Atopic dermatitis: New insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000;105:860–876.
- 11 Kingetsu I, Ohno N, Hayashi N, Sakaguchi M, Inoue S, Saito S: Common antigenicity between Japanese cedar (*Cryptomeria japonica*) pollen and Japanese cypress (*Chamaecyparis obtusa*) pollen. I. H-2 complex affects cross responsiveness to Cry j1 and Cha o1 at the T- and B-cell level in mice. *Immunology* 2000;99:625–629.
- 12 Enomoto T, Ohnishi S, Dake Y, Sakota T, Saitoh Y, Sogo H, Seno S, Fujiki Y, Fujimura S: Japanese cedar pollen in house dust (in Japanese). *Arerugi* 2001;50:535–539.
- 13 Miles EA, Warner JA, Jones AC, Colwell BM, Bryant TN, Warner JO: Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents. *Clin Exp Allergy* 1996;26:780–788.
- 14 Piccinni MP, Mecacci F, Sampognaro S, Manetti R, Parronchi P, Maggi E, Romagnani S: Aeroallergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993;102:301–303.
- 15 Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, Holt PG: Transplacental priming of the human immune system to environmental allergens: Universal skewing of the initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730–4737.
- 16 Szépfalusi Z, Pichler J, Elsässer S, van Duren K, Ebner C, Bernaschek G, Urbanek R: Transplacental priming of the human immune system with environmental allergens can occur early in gestation. *J Allergy Clin Immunol* 2000;106:530–536.