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ORIGINAL ARTICLE

Survey of Food and Airborne Allergen-specific IgE Levels in a General Population of 3-year-old Japanese Children

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ABSTRACT

Background: Background data on the allergic constitutions of a general population of Japanese children may provide important information regarding environmental factors that contribute to the increased incidence of allergic diseases and suggest clues for their prevention.

Methods: Serum samples were obtained from a general population of 3-year-old children (612 samples) in the Kanto area and Asahikawa city. The serum levels of total and specific IgE antibodies against food allergens (egg white, milk, soybean, wheat), indoor airborne allergens (house dust, mite, cat dandruff), and outdoor airborne allergens (Japanese cedar or white birch pollen allergens) were determined and analyzed, along with the results of a questionnaire regarding medical history, and allergy-related subjective symptoms.

Results: The mean total IgE level of all subjects was 34.7 IU/ml, while that for the Kanto and Asahikawa areas was 44.7 and 22.5 IU/ml, respectively. Twenty six percent of the 612 children were judged positive for indoor airborne allergen-specific IgE, and 6.7% were positive for food allergen-specific IgE. Cedar allergen-specific IgE was detected in 15.6% of the children living in the Kanto area. The total IgE level was strongly correlated with the number of allergens to which the child was sensitized.

Conclusions: A relatively large number (28.4%) of 3-year-old children possessed allergen-specific IgE antibodies. The basic data obtained in the general population in these two areas of Japan will be valuable for further evaluations of environmental influences on allergic disease.

KEY WORDS

3-year-old children, antigen-specific IgE, pollen, serum, total IgE

INTRODUCTION

The incidence of allergic diseases in children in industrialized countries has recently been increasing,[1] and 80% of atopic infants under 12 months of age in Japan are reported to be positive for food allergen-specific immunoglobulin E (IgE) antibodies, mostly for egg white-specific IgE.[2] It has been hypothesized that most allergic children are sensitized to food allergens first, then gradually to indoor airborne allergens, and finally to outdoor airborne allergens, such as pollen allergens.[1] However, a recent report revealed that Japanese cedar allergen-specific IgE had been detected even in children under 12 months of age and that some of them had not been sensitized to any food and/or indoor airborne allergens.[3]

In this study we conducted a survey on the incidence of allergy in 3-year-old children by determining the serum total and specific IgE levels of 612 children in the metropolitan Kanto area and in Asahikawa city in Hokkaido (the northernmost of the major islands of Japan). The environment in the Kanto area is that of a typical city, and children are exposed to relatively polluted air, and to Japanese cedar pollen in the early

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Table 1 Average serum total IgE levels and frequency of children positive for specific IgE to seven major allergens

Year	Area	No. of samples	Allergic incidence of parents†	Total IgE (IU/ml) geometric mean ± SD	Specific IgE (frequency)		
					Indoor airborne allergens‡	Food allergens§	At least 1 of the 7 allergens
2000	Kanto¶	74	47 (63.5%)	58.5 ± 5.6	18 (24.3%)	12 (16.2%)	27 (36.5%)
2001	Kanto	189	132 (69.8%)	39.5 ± 4.9	53 (28.0%)	16 (8.5%)	58 (30.7%)
2002	Kanto	78	52 (66.7%)	45.7 ± 4.3[a]	22 (28.2%)	3 (3.8%)	24 (30.4%)
	Asahikawa	126	64 (50.8%)	19.4 ± 5.5[b]	32 (25.4%)	4 (3.2%)	32 (25.4%)
2003	Kanto	45	28 (62.2%)	46.9 ± 5.3	7 (15.6%)	2 (4.4%)	7 (15.6%)
	Asahikawa	100	66 (66.0%)	27.2 ± 5.0	26 (26.0%)	4 (4.0%)	26 (26.0%)
2000–2001	Kanto	263	179 (68.1%)	44.1 ± 5.1	71 (27.0%)	28 (10.6%)	85 (32.3%)
2002–2003	Kanto	123	80 (65.0%)	46.1 ± 4.6[c]	29 (23.6%)	5 (4.1%)	31 (25.2%)
2002–2003	Asahikawa	226	130 (57.5%)	22.5 ± 5.3[d]	58 (25.7%)	8 (3.5%)	58 (25.7%)
2000–2003	Total	612	339 (63.6%)	34.7 ± 5.2	158 (25.8%)	41 (6.7%)	174 (28.4%)

†Frequency of the children whose mother and/or father had allergic history
‡Any 1 of house dust, mite, and cat dandruff
§Any 1 of wheat, soybean, milk and egg white
¶Kanto area includes Higashikurume, Tama, and Yokohama
a-b, c-d: $p < 0.001$; Mann-Whitney's *U*-test

spring. Asahikawa is also a relatively large city, but it is located near Taisetuzan National Park, and its population is not as dense as that in the Kanto area. Since the climate is cooler than in the Kanto area, Japanese cedar trees do not grow in Asahikawa city and the incidence of allergy to white birch pollen has been increasing instead.

We chose four food allergens (egg white, milk, soybean, and wheat), and three indoor airborne allergens (house dust, mite [*Dermatophagoides farinae*], and cat dandruff) to survey the serum of 3-year-old children for allergen specific-IgE levels. These are the most common allergens to which Japanese children are exposed. Furthermore, serum levels of Japanese cedar and white birch pollen allergen specific-IgE were also determined in children living in the Kanto area and Asahikawa city, respectively. Serum data and the results of a questionnaire concerning allergic symptoms were then analyzed together.

METHODS

SUBJECTS AND SERA

Serum specimens were obtained from 612 children, who underwent routine medical checkups for 3-year-old children by the local governments of Higashikurume city and Tama city in Tokyo prefecture, and the Asahi ward of Yokohama city in Kanagawa prefecture, which are in the metropolitan Kanto area, and Asahikawa city in Hokkaido from October to January during the 4 years from 2000 to 2003. We requested all the subjects of the medical checkups to participate in this study and more than half consented. Blood samples were available from 45–55% of all subjects. This study received ethical approval from the Institutional Review Board of Asahikawa Medical College and the National Institute of Health Sciences.

The numbers of subjects in each area and each year are shown in Table 1. The subjects were enrolled in the study after obtaining informed consent to voluntary participation in this study and to its purpose from their parents. Peripheral blood was collected from the participants by venipuncture, and at the same time, the parents were requested to fill out a questionnaire concerning their child's symptoms, previous diagnosis, parents' history of allergy, life style, and living environment. Blood samples from healthy adult volunteers were used as controls after obtaining informed consent. The serum prepared from the blood samples was maintained at –20℃ until tested.

DETERMINATION OF IgE ANTIBODIES

Total IgE antibodies were determined using the Pharmacia CAP System™ IgE fluorescence enzyme immunoassay (FEIA, Pharmacia, Uppsala, Sweden). The detected value was shown as international units (IU)/ml, and the detection limit was 2 IU/ml.

Allergen-specific antibodies were determined by immunoassay with a Quidel Allergen Screen II kit (QAS II, Xenith Biomed, Ireland) according to the manufacturer's instructions. We chose the most common allergens among Japanese children: four food allergens (egg white, milk, soybean, and wheat), three indoor airborne allergens (house dust [house dust 1, Greer Labs. Inc., Lenoir, NC, USA], mite [*Dermatophagoides farinae*], and cat dandruff). The kit consists

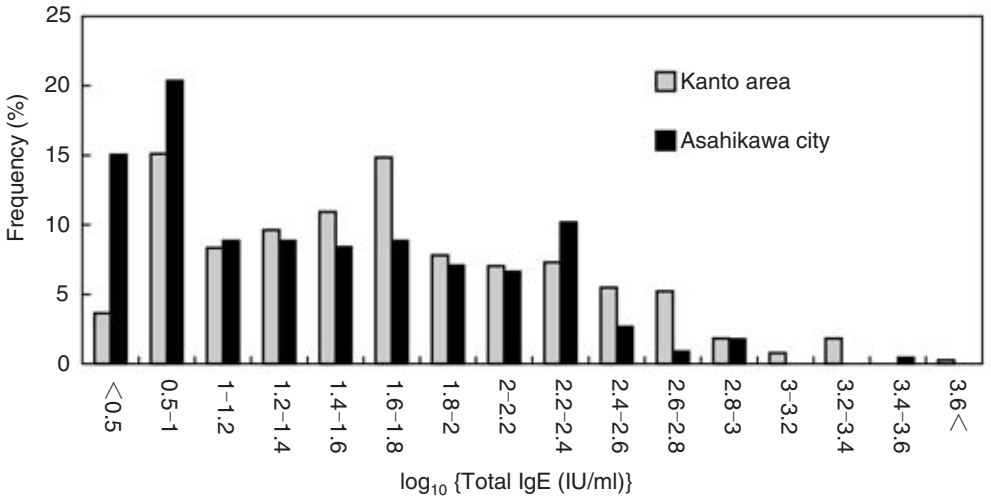

Fig. 1 Histogram of total IgE levels. The total IgE levels of 386 children in the Kanto area and 226 children in Asahikawa city have been plotted. The logarithms of the IgE concentrations are shown along the abscissa, and the frequency of children along the ordinate.

of a stick to which 7 paper pads coated with a different allergen and a negative control pad are attached. The results were classified into 6 grades: 0, negative; ±,possibly positive; and 1–4, depending on the absorbance of the colored pads after reaction. The grading method was previously confirmed to correlate with radioallergosorbent test (RAST) scores,[4,5] and serum judged to be positive with the QAS kit has been suggested to correspond to a RAST score of more than 2.

Japanese cedar pollen Cry j 1- and Cry j 2-specific IgE levels were determined by an ELISA. Assay plates (96 wells) were coated with purified Cry j 1 or Cry j 2 (Code No. HBL-C-1 and HBL-C-2, respectively; Hayashibara Biochemical Laboratories, Inc., Okayama, Japan; 0.2 μg/50 μl/well) and incubated at 4℃ overnight. After washing, the plates were blocked with phosphate-buffered saline (PBS, pH 7.0) containing 0.1% casein for 2 hours at room temperature and then incubated with serum samples diluted to 5% in 0.1% casein-PBS at 4℃ overnight. The plates were then sequentially treated with rabbit anti-human IgE antibodies (RAHu/IgE[Fc]; Nordic Immunology, Stockholm, Sweden) and peroxidase-conjugated anti-rabbit Ig (NA 9340; Amersham Biosciences, Piscataway, NJ, USA), and then reacted with a colorimetric substrate solution (TMB reagent, Cat. No. 555214; BD Biosciences, San Diego, CA, USA). Colorimetric intensity (OD450–570) was compared with that of the serum from 6 healthy donors, and samples of which the absorbance was greater than the average value plus five times the standard deviation of the healthy donors were judged to be positive.

White birch allergen-specific IgE levels were determined with a liquid phase inhibitor assay (AlaSTAT; DPC Biermann, Los Angeles, CA, USA).

RESULTS

TOTAL IgE LEVELS

The average values (geometric means) for the total IgE detected in each area and year are shown in Table 1. The overall average (n = 612) was 34.7 IU/ml. In Higashikurume city, samples were available each year during the 4-year period of the study, and the IgE level of the samples obtained in 2000 was somewhat higher than that in the subsequent 3 years. The average total IgE level in the Kanto area from 2000 to 2003 was 44.7 IU/ml, and it was 22.5 IU/ml in Asahikawa city in the final 2 years of the study (2002 and 2003), about half the level in the Tokyo area, and the difference was statistically significant ($p < 0.001$; Mann-Whitney's U test). The distribution of the total IgE values in the Kanto area and Asahikawa city is shown in the form of a histogram in Figure 1. In Asahikawa city, 34.1% of the samples contained very low IgE levels (lower than 10 IU/ml), and this percentage was significantly higher than in the Kanto area (16.9%; odds ratio: 3.13, 95% confidence interval [CI]: 2.13–4.61). By contrast, the percentage of children with levels of ≧200 IU/ml was twice as high in the Kanto area (20.1%) as in Asahikawa city (9.7%; odds ratio: 2.31, 95% CI: 1.39–3.83).

LEVELS OF IgE SPECIFIC FOR FOOD AND INDOOR AIRBORNE ALLERGENS

The numbers of positive samples for specific IgE in each year and in each area are also shown in Table 1. The proportion of sera positive for at least one of the 7 allergens was 28.4%. Overall, 25.8% of the samples were positive for indoor airborne allergens and the

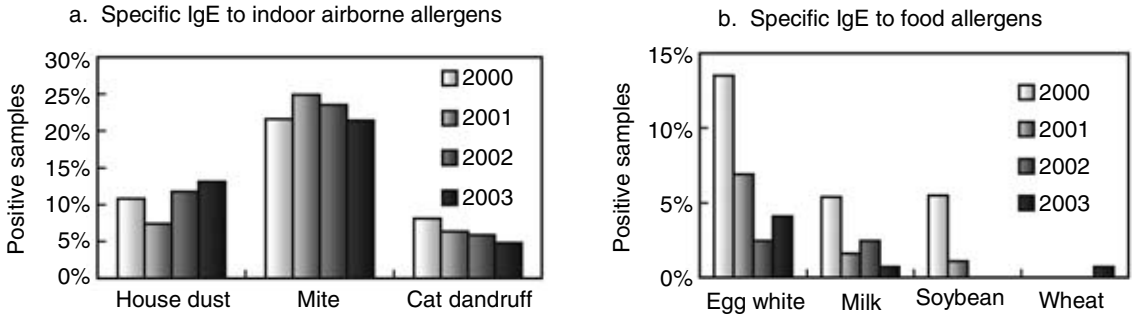

Fig. 2 Ratios of specific IgE antibody-positive samples for seven major allergens over a 4-year period. (**a**) Indoor airborne allergens. (**b**) Food allergens. Sera from 612 children were analyzed using a QAS II kit. The positive ratio for each allergen was plotted.

Table 2 Relationship between specific IgE detection and allergic symptoms

Area	Specific IgE†	No. of samples	Children with allergic symptoms‡		Children with a diagnosis of allergy§	
Kanto	Positive	116	57	49.1%	63	54.3%
	Negative	270	88	32.6%	58	21.5%
	Total	386	145	37.6%	121	31.3%
Asahikawa	Positive	58	33	56.9%	34	58.6%
	Negative	168	70	41.7%	64	38.1%
	Total	226	103	45.6%	98	43.4%
Total	Positive	174	90	51.7%	97	55.7%
	Negative	438	158	36.1%	122	27.9%
	Total	612	248	40.5%	219	35.8%

The numbers of children and percentages of specific IgE-positive or negative children are shown.
†specific IgE antibodies to any of the seven major allergens as determined by QAS II
‡subjective allergic symptoms: wheezing, rhinitis, conjunctivitis
§diagnosis: allergic asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, food allergy

frequency was not significantly different among the four years. As shown in Figure 2, the allergen to which the largest proportion of children had specific IgE was mites (23.2% on the average). In addition, the QAS scores for mite-allergen positive sera were higher than those for sera positive for the other allergens (data not shown). The second most common allergen was house dust (10.6% on the average), although the scores were lower than those for mites and cat dandruff.

Overall, 6.7% of the sera were positive for food allergens. The number of children sensitized to each food allergen was in the order of egg white, milk, soybean, and wheat. The proportion of sera positive for food allergens was higher in the Kanto area than in Asahikawa city (Table 1, odds ratio: 2.55, 95% CI: 1.16–5.62), but the proportion of sera positive for indoor airborne allergens was not significantly different between the two areas.

RELATIONSHIP BETWEEN ALLERGEN-SPECIFIC IgE AND THE ALLERGIC STATUS OF CHILDREN

The relationship between subjective allergic symptoms and diagnoses, and allergen-specific IgE was analyzed (Table 2). Allergic symptoms were present in 51.7% of the specific IgE-positive children, and 55.7% had been previously diagnosed with an allergic disease. Thus, about half of the children with allergen-specific IgE had no allergic symptoms. By contrast, 36.1% of the specific IgE-negative children had allergic symptoms, and 27.9% had been diagnosed with an allergic disease.

RELATIONSHIP BETWEEN TOTAL IgE LEVEL AND PRESENCE OF ANTIGEN-SPECIFIC IgE

The relationships between total IgE levels and the numbers of allergens to which the serum IgE was positive are shown in Figure 3. As expected, the children with higher levels of total IgE were positive for IgE to more allergens.

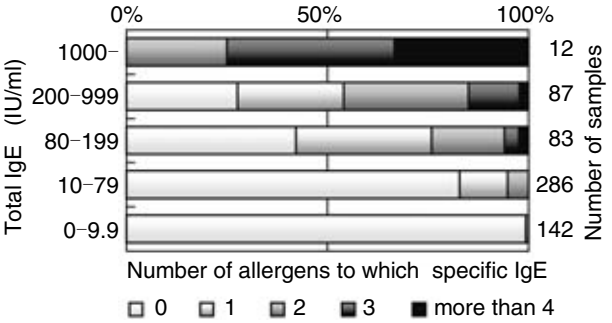

Fig. 3 Relationship between total IgE level and the number of reacting allergens among the 7 major allergens tested with a QAS II kit. Serum samples were categorized into five groups according to their total IgE level, and the number of reacting allergens (house dust, mite, cat dandruff, egg white, milk, soybean, and wheat) was counted.

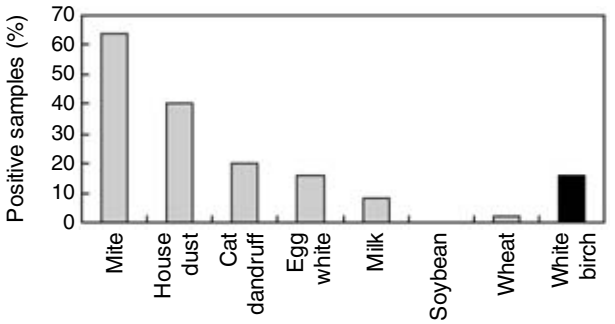

Fig. 4 Ratios of specific IgE antibody-positive samples for seven major allergens and white birch pollen over a 2-year period (2002 – 2003) in children with high total IgE value (≧ 100 IU/ml) in Asahikawa city.

SPECIFIC IgE TO POLLEN ALLERGENS

Specific IgE antibodies to Cry j 1 and Cry j 2, which are the major allergens of Japanese cedar pollen, were detected in the sera from the Tokyo area. Of the 307 samples analyzed, 13.7% were positive for IgE to Cry j 1 and 4.6% were positive for IgE to Cry j 2. Overall, 15.6% of the children were positive for Cry j 1- and/or Cry j 2-specific IgE, but most of them did not show any symptoms of pollen allergy.

The odds ratio between the high (≧100 IU/ml) and low (<100 IU/ml) total IgE groups for food allergen- and indoor airborne allergen-positive children was 6.60 and 12.49, respectively, but was only 2.42 for Cry j 1 and/or Cry j 2-positive children (Table 3).

Children with high total IgE values in Asahikawa city were tested for white birch pollen-specific IgE antibodies, and 8 of the 50 sera (16%) were positive (Fig. 4), however the IgE levels of positive samples were relatively low (AlaSTAT score 1–2).

DISCUSSION

Only a few previous studies in Japan have targeted very young children in the general population and involved blood sampling. We determined the total IgE and the allergen-specific-IgE levels in the sera of 3-year-old children in order to obtain basic data in the general population and to evaluate environmental influences on allergic disease. The ratio of 0- to 4-year-old children with allergic symptoms was reported to be 37.3% in 2003 by the Ministry of Health, Labor and Welfare of Japan and that of 3-year-old children was 41.9% in 1999 by the Tokyo prefectural government, which was similar to that of the subjects of this trial (Table 2).

The mean of the total IgE levels of all subjects (612 children) was 34.7 IU/ml, and there was a significant difference between the total IgE levels in the Tokyo area and those in Asahikawa city (44.7 and 22.5 IU/ml, respectively). The difference in proportion of subjects with very low IgE levels (<10 IU/ml) was thought to be the major reason for the difference in mean total IgE level between the Tokyo area and Asahikawa city.

The ordinary range of serum total IgE levels varies with age and the population. According to Pharmacia Diagnostics (Uppsala, Sweden), the expected total IgE level of 3-year-old children is 8 IU/ml (geometric mean), and the mean + 1 SD is 32 IU/ml.[6] Naspitz *et al.* reported that the total IgE levels in non-allergic Brazilian children 2–3 years old and 3–4 years old were 26.4 and 69.3 IU/ml, respectively.[7]

In Japan, Morikawa[8] reported that most children 2–3 years old, whose total IgE level was 15 IU/ml or lower, were negative for major allergens in the RAST, and the low IgE group was expected to maintain a low IgE level in the future. On the other hand, more than 90% of the children with an IgE of 40 IU/ml or more were RAST-positive. Since the children enrolled in the above studies had been examined for suspected allergic disease, the above results cannot be directly compared with the data obtained in our study. Morikawa also proposed threshold ranges of total IgE levels for estimation of future allergic risk of 15–40 and 30–70 IU/ml for children 2–3 years and 3–5 years of age, respectively. The proportion of children whose IgE levels were in the threshold ranges was higher in the Kanto area than in Asahikawa city (Fig. 1).

The indoor airborne allergen-positive ratios in the two areas did not differ significantly (Table 1; Kanto area: 25.9%, Asahikawa city: 25.7%) and this is the first report about the frequency of children positive for indoor allergen-specific IgE in the general population. Although there were differences in the positive ratios for IgE to food allergens between the two areas, and in the Kanto area during 2000 to 2003 (Table 1, Fig. 2b), there were not enough positive cases to test for

Table 3 Relationship between total IgE levels and detection of specific IgE in Kanto area samples

Total IgE (IU/ml)	No. of samples	Specific IgE-positive samples		
		Food allergens	Indoor airborne allergens	Cry j 1 and/or Cry j 2
≧100	89	14 (15.7%)	55 (61.8%)	22 (24.7%)
＜100	218	6 (2.8%)	25 (11.5%)	26 (11.9%)
Total	307	20 (6.5%)	80 (26.1%)	48 (15.6%)
Odds ratio (95% CI)		6.60 (2.45–17.78)	12.49 (6.78–22.69)	2.42 (1.29–4.56)

the statistical significance. We found nothing in the results of the questionnaire or other serum data that could explain this phenomenon.

The percentage of the population with food allergy has been reported to be 1–2% of adults and 6–8% of children,[9,10] and the results of the present study (6.7%) were within the range for children. In the Kanto area, 75% of the children positive for food allergen-specific IgE had also been sensitized to indoor airborne allergens or cedar pollen allergens. It is likely that these children were sensitized to food allergens during infancy, that the sensitization persisted until they were 3 years old, and that they then acquired indoor or outdoor airborne allergen-specific IgE. The number of persons with wheat allergen-specific allergy has been reported to be increasing in Japan.[11,12] One of the 612 subjects in our study was judged to be positive for specific IgE to wheat allergens, and possessed IgE to all allergens tested except soybean allergens.

Cedar pollen allergen-specific IgE was determined by ELISA, which was a different method than that used with other allergen-specific IgE, but the samples judged to be positive by QAS cedar pollen allergen-coated pad were confirmed to be positive for Cry j 1 or Cry j 2 by ELISA (data not shown). No clear difference in the proportion of children positive for IgE to Japanese cedar pollen allergens was seen between the high and low total IgE groups (Table 3). Six children were positive despite low total IgE levels (less than 20 IU/ml). Among the 307 children who were examined for cedar pollen allergen-specific IgE, 48 were positive, and 47.9% (23 children) were positive for IgE to cedar pollen alone.

Kulig *et al.* reported that 6–7% of 3-year-old children in Germany had been sensitized to birch pollen and timothy grass pollen, and that 3% had been diagnosed with seasonal allergic rhinitis.[13] The results of the present study showed that the percentage (15.6%) of children in the Kanto area who were sensitized to cedar pollen allergen-specific IgE was much higher, but only a few had symptoms of pollen allergy. It has been reported that the age of sensitization to cedar pollen is decreasing, and once the pollen-specific IgE antibodies are possessed by children, they hardly decrease.[3] Thus, IgE-positive children are at risk of developing pollen-allergic symptoms in the future.

The children who were positive for specific IgE tended to exhibit allergic symptoms (Table 2), and the total IgE level was strongly correlated with the numbers of allergens to which they had been sensitized (Fig. 3). Thus, the total IgE value seemed to be a good indicator of allergic status. However, some children with low total IgE levels and/or children negative for allergen-specific IgE exhibited allergic symptoms or had been given a diagnosis of an allergic state. Some explanations may be possible as follows: sensitization to minor allergens other than the 7 allergens tested here, causing allergic reactions by very low levels of specific IgE; or existence of IgE-independent allergies.

Seventy percent of the children with mild allergic symptoms are thought to experience remission of their allergies by puberty, but recovery is rare in children with severe allergy.[2] Therefore, it is important to know the existing allergic state of very young children and identify allergic factors. The present study clarified the actual levels of total IgE and of specific IgE to major allergens in 3-year-old Japanese children. It will provide useful information for establishing measures to prevent the worsening of allergic conditions. Furthermore, regional differences in total and specific IgE levels were demonstrated which most probably reflect environmental differences between the areas.

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