Development of antigen-specific IgE antibodies in atopic and non-atopic infants: Diagnostic value of low levels of IgE against egg white in infants with atopic dermatitis

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ABSTRACT
To analyze the development of antigen-specific IgE in infants and its clinical usefulness, the levels of IgE antibodies against egg white (f1) and cows' milk (f2) in 33 sera from cord blood, 118 sera from atopic dermatitis (AD) infants and 197 sera from non-atopic control infants were measured by using the CAP radioallergosorbent test (RAST) fluoro enzyme immunoassay (FEIA) system, which has a detection limit of 0.15 U/mL for f1 and 0.20 U/mL for f2. No antigen-specific IgE was detected in cord blood, whereas in infants younger than 6 months of age, 38.5% of AD infants and 6.6% of the non-atopic controls showed IgE against f1 (~0.70 U/mL) and 14.3 and 4.0%, respectively, showed low levels (0.15-0.70 U/mL) of the IgE. When the cut-off point for positive versus negative f1 RAST was set at 0.15 or 0.35 U/mL instead of 0.70 U/mL, the significance of the difference in f1 RAST-positive and -negative proportions between atopic and non-atopic infants did not change. Repeated examination of f1 RAST revealed later positive conversion in the majority of AD patients, with no detectable or very low levels (0.15-0.35 U/mL) of f1-specific IgE. In infants at 6 months of age or older, 44.4 and 12.0% of AD patients and non-atopic controls, respectively, showed IgE against f1 (≥0.70 U/mL) and 37.1 and 22.6%, respectively, showed low levels (0.15–0.70 U/mL) of the IgE. These results suggest that f1 RAST at a concentration of 0.15 U/mL or higher has diagnostic value for egg allergy in AD infants, especially in infants younger than 6 months of age. The f1 RAST should be examined repeatedly in AD infants with low levels of IgE against f1. Similar results were obtained for f2-specific IgE, but there was a significant decrease in the specificity when the cut-off point was set at 0.20 U/mL.

Key words: atopic dermatitis, egg, infant, milk, RAST.

INTRODUCTION
IgE plays an important role in the development of atopic diseases, such as allergic rhinitis, bronchial asthma and atopic dermatitis (AD). Although the role of IgE in AD has not yet been fully clarified, antigen-specific IgE antibodies have been detected in a large proportion of infants and young children with AD. Among antigen-specific IgE, egg white-specific IgE is the most frequently detected in infants with AD in Japan and cows' milk-specific IgE is the next most frequent.1,2

Atopic dermatitis in children usually occurs at a very early time of life. In a previous study, 47 and 72% of children with AD developed symptoms within the first 6 and 12 months after birth, respectively.3 However, the diagnosis of AD is sometimes difficult in the early infantile period because its chronic course has not yet been established. In this situation, the detection of antigen-specific IgE in the serum of the infant is helpful,
although not completely specific in the diagnosis of AD and determination of the antigens. Early detection of food allergy and elimination of the responsible food may prevent the later development of other food allergy and respiratory atopy, although this is still controversial. However, we have often encountered infants with AD without any positive antigen-specific IgE who later showed high levels of food-specific IgE, as was also found in the present study.

An antigen-specific IgE antibody concentration lower than 0.35 Ua/mL (RAST score 0) determined by the radioallergosorbent test (RAST) has been considered negative, whereas a RAST score of 2 or higher has been considered positive in the general population. However, considering the facts that AD first appears in the early infantile period and that the serum IgE level is extremely low at birth and increases during childhood, a RAST score of 1 is sometimes considered positive in infants. This suggests that antigen-specific IgE at concentrations lower than 0.35 Ua/mL may also have a diagnostic value.

To clarify the early development of antigen-specific IgE and the clinical usefulness of its low levels in AD, we examined serum antigen-specific IgE in cord blood, in AD infants and in age-matched, non-atopic controls. We found that low levels (0.15-0.70 Ua/mL) of IgE against f1 have a diagnostic value in AD infants.

**METHODS**

**Patients**

Serum was obtained from AD infants and non-atopic control infants who visited the Departments of Pediatrics at Kyoto University Hospital and at 15 affiliated hospitals in eight prefectures in Japan between February 1995 and March 1996. The AD group consisted of 118 infants (74 boys) with a mean (±1 SD) age of 4 ± 2 months (range 1-11 months). The non-AD control group consisted of 197 infants (105 boys) with a mean age of 5 ± 4 months (range 0-11 months) who visited the hospitals for health checkups or had some disease other than AD, bronchial asthma or any other disease that was considered to influence IgE production. Subjects were selected randomly using a computer to match for age (month) and sex. Sera were kept at -40°C until analysis. Ninety-one percent of infants with AD were being breast fed (53% breast fed; 38% mixed feeding with breast milk and cows’ milk) and 9% of these infants had only cows’ milk at the time of examination, although the majority of them had also had breast milk for various periods before the examination. There was no significant difference in the mode of feeding between the AD and non-atopic groups. Cord serum from 33 normal full-term deliveries was also obtained. Informed consent was obtained from the parents or the guardians.

**Diagnosis of AD**

Atopic dermatitis was defined as a pruritic and chronic or chronically relapsing dermatitis with typical features and distribution. Because the chronic course of AD (one of the criteria for diagnosis) had not been established in many patients during the early infantile period, the diagnosis of AD was confirmed by repeated examinations in almost all patients at the earliest 1 month after blood sampling. The diagnosis of AD was not made in the case of a patient with eczema, which disappeared easily within a few weeks of only keeping the skin clean with or without topical corticosteroids, antibiotics or anti-fungal reagents and did not appear again after cessation of the therapy.

**Detection of serum antigen-specific IgE**

Serum levels of specific IgE antibodies against egg white (f1), cows’ milk (f2), soy bean (f14), and mites (d1) were determined using the CAP RAST FEIA system (Pharmacia Upjohn Diagnostics, Tokyo, Japan) according to the manufacturer’s instructions.

**STATISTICAL METHODS**

Data were analyzed using the repeated χ² test with or without Bonferroni’s correction.

**RESULTS**

**Sensitivity of the CAP RAST system**

We first examined the sensitivity of the CAP RAST FEIA system. The lowest detectable concentration of IgE determined as the 0 standard + 3 SD (n = 6) was 0.04-0.05 Ua/mL when solution buffer was used as the 0 standard (data not shown). A dilution assay using serum containing known levels of specific IgE against f1, f2, f14 or d1 revealed that this assay system satisfactorily detected serum IgE against these antigens at concentrations below 0.35 Ua/mL (Fig. 1). Examination for reproducibility in
the measurement of antigen-specific IgE at very low concentrations revealed that the CAP RAST system measured serum IgE at the lowest concentrations of 0.15 Ua/mL for f1 and 0.20 Ua/mL for f2, f14 and d1 with intra- and interassay coefficients of variation (CV; n = 3) of less than 10% (data not shown).

**Antigen-specific IgE in sera from cord blood, infants with AD and non-atopic controls**

We examined the serum levels of specific IgE against egg white (f1), cows' milk (f2), soy bean (f14) and mites (d1) in sera from cord blood, AD infants and non-atopic controls.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1/1</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
</tr>
</thead>
<tbody>
<tr>
<td>[IgE]</td>
<td>1.83</td>
<td>0.87</td>
<td>0.5</td>
<td>0.25</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>CV</td>
<td>5.1</td>
<td>9.6</td>
<td>1.7</td>
<td>4.7</td>
<td>8.7</td>
<td>37.4</td>
</tr>
</tbody>
</table>

**Fig. 1** Sensitivity of the CAP RAST FEIA system for low levels of specific IgE (Ua/mL) against (a) f1, (b) f2, (c) f14 and (d) d1. Serum containing a known level of specific IgE antibody against f1, f2, f14 or d1 was diluted with dilution buffer and the level of specific IgE against these antigens was examined with the CAP RAST FEIA system to determine the sensitivity of the system for very low levels of specific IgE. Linearity for dilution is shown by representative data from three reproducible experiments with the use of different sera. When the serum was diluted with a standard serum containing no IgE instead of dilution buffer, the results were the same (data not shown). CV, coefficient of variation (n=3).
None of the cord sera examined (n = 33) contained detectable levels (≥ 0.15 Ua/mL for f1 and ≥ 0.20 Ua/mL for f2, f14 and d1) of specific IgE against these antigens, whereas 59.3 and 36.8% of AD infants showed detectable levels of specific IgE against f1 and f2, respectively (Fig. 2; Table 1). In the present study, there were only two and one AD infants with detectable IgE against f14 and d1, respectively.

When the cut-off point for positive versus negative f1 RAST was set at 0.70 Ua/mL (class 2), the proportion of positive AD infants and non-atopic controls was 38.5 and 6.6%, respectively, in infants younger than 6 months of age and 44.4 and 12.0%, respectively, in infants 6 months of age or older. When the cut-off point of f1 RAST was set at 0.35 Ua/mL (class 1) or at 0.15 Ua/mL, the proportion of f1 RAST-positive AD infants further increased (Table 1). Although the proportions of non-atopic controls with IgE against f1 (≥ 0.35 or ≥ 0.15 Ua/mL) were considerably large, they were still much smaller than those of AD infants, particularly in infants younger than 6 months of age. The correlation between age and f1 RAST in infants is shown in Fig. 3.

IgE against f2 showed similar results (Figs 2, 3). When the cut-off point of f2 RAST was set at 0.70 Ua/mL, the proportion of positive atopic patients and non-atopic controls was 18.9 and 6.6%, respectively, in infants younger than 6 months of age and 33.3 and 5.3%, respectively, in infants 6 months of age or older. When the cut-off point of f2 RAST was set at 0.35 Ua/mL, the proportion of positive atopic patients and non-atopic controls was 28.9 and 9.0%, respectively, in infants younger than 6 months of age and 40.7 and 12.9%, respectively, in infants 6 months of age or older. When

![Graph showing egg white- and cows' milk-specific IgE in cord blood (CB) and in infants. (a) F1 RAST; (b) F2 RAST. NOR, non-atopic control infants; AD, infants with atopic dermatitis.](https://example.com/graph.png)
### Table 1. IgE against f1 and f2 in infants

<table>
<thead>
<tr>
<th>IgE level (Ua/mL)</th>
<th>Age &lt; 6 months</th>
<th>Proportion of infants (%)</th>
<th>Age ≥ 6 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOR (n=122)</td>
<td>AD (n=91)</td>
<td>NOR (n=75)</td>
<td>AD (n=27)</td>
</tr>
<tr>
<td>f1 RAST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.15</td>
<td>89.4</td>
<td>47.2</td>
<td>65.4</td>
<td>18.5</td>
</tr>
<tr>
<td>≥ 0.15</td>
<td>10.6</td>
<td>52.8</td>
<td>34.6</td>
<td>81.5</td>
</tr>
<tr>
<td>&lt;0.35</td>
<td>91.0</td>
<td>57.1</td>
<td>74.7</td>
<td>40.7</td>
</tr>
<tr>
<td>≥ 0.35</td>
<td>9.0</td>
<td>42.9</td>
<td>25.3</td>
<td>59.3</td>
</tr>
<tr>
<td></td>
<td>&lt;0.20</td>
<td>81.2</td>
<td>81.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 0.20</td>
<td>18.8</td>
<td>18.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.35</td>
<td>91.0</td>
<td>87.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 0.35</td>
<td>9.0</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>f2 RAST</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;0.20</td>
<td>81.2</td>
<td>65.6</td>
<td>81.3</td>
<td>55.6</td>
</tr>
<tr>
<td>≥ 0.20</td>
<td>18.8</td>
<td>34.4</td>
<td>18.7</td>
<td>44.4</td>
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<tr>
<td>&lt;0.35</td>
<td>91.0</td>
<td>71.1</td>
<td>87.1</td>
<td>59.3</td>
</tr>
<tr>
<td>≥ 0.35</td>
<td>9.0</td>
<td>28.9</td>
<td>12.9</td>
<td>40.7</td>
</tr>
</tbody>
</table>

NOR, non-atopic control infants; AD, infants with atopic dermatitis. *P<0.05, **P<0.01.

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**Fig. 3** Egg white- and cows’ milk-specific IgE in infants. (a) f1 RAST; (b) f2 RAST. Serum levels of IgE against egg white and cows’ milk in infants with AD (AD) and non-atopic control infants (NOR) are shown according to their ages in months. The figures in the bars are the percentages and the number of subjects are shown in parentheses at the bottom of each column. (☐), ≥ 0.35 Ua/mL; (■), 0.15–0.35 Ua/mL in (a) and 0.20–0.35 Ua/mL in (b); (□), <0.15 Ua/mL in (a) and <0.20 Ua/mL in (b).
the cut-off point was changed from 0.35 to 0.20 Ua/mL, the increase in the proportion of f2 RAST-positive AD patients (5.2%) was smaller than that in the non-atopic controls (8.6%), with a decrease in the significant difference in distribution of f2 RAST-positive and -negative subjects between atopic and non-atopic infants (Table 1).

Serial change in f1 RAST levels in infants with AD

The f1 RAST was examined repeatedly in 41 AD infants (Fig. 4). Fourteen of 18 patients (77.8%) at 2-4 months of age without detectable IgE against f1 at the time of first examination later showed IgE against f1 at a concentration of 0.35 Ua/mL or higher. Four of five AD infants (80%) with IgE against f1 in the concentration range 0.15–0.35 Ua/mL showed f1 RAST at a concentration of 0.35 Ua/mL or higher at the time of re-examination. In contrast, 10 of 18 AD infants (55.6%) with f1 RAST at a concentration of 0.35 Ua/mL or higher showed decreased levels of f1 RAST at the time of re-examination within 1 year.

DISCUSSION

IgE against egg white is the most frequently seen antigen-specific IgE in Japanese infants with AD. Although the role of IgE in the pathogenesis of AD has not yet been fully elucidated, egg allergy plays a central role in the majority of Japanese infants with AD. In more than 80% of AD infants with IgE against egg white, elimination and loading tests have revealed that egg white is the responsible allergen (S Ito et al., unpubl. obs., 1993). Therefore, IgE against egg white is considered to be important in the detection of allergens in AD infants.

In the present study we detected no IgE against egg white, cows’ milk, soy bean or mites in sera from cord blood using a sensitive CAP RAST FEIA system and IgE against egg white or cows’ milk gradually became detectable during the early infantile period in AD patients and, much less frequently, in non-atopic infants. This is in good agreement with earlier reports (reviewed by Zeiger). Thus, the early infantile period is the period in which antigen-specific IgE antibodies mainly develop in atopic patients and it is to be expected that low levels of IgE against egg white have some diagnostic value for egg allergy in infants, particularly in infants younger than 6 months of age.

Determination of the cut-off point of antigen-specific IgE is difficult; when the cut-off point is set at a high concentration, the sensitivity may decrease even though the specificity may increase. Conversely, when the cut-off point is set at a low concentration, the specificity may decrease even though the sensitivity may increase. In the present study, the sensitivity increased but the specificity decreased when the cut-off point for f1 RAST was set at 0.35 or 0.15 Ua/mL instead of 0.75 Ua/mL; considerably large proportions (15.2 and 19.8%, respectively) of non-atopic control infants, even including those younger than 6 months of age, were positive for egg white-specific IgE when the cut-off points were set at 0.35 and 0.15 Ua/mL. As far as the specificity is concerned, this is a disadvantage of low cut-off points. However, even when the cut-off point was set at 0.70 Ua/mL, there was still a considerable proportion (8.6%) of positivity for f1 RAST in non-atopic controls. It is apparent that IgE
RAST cannot completely diagnose allergy by itself and that the determination of food allergens in children with AD by serum antigen-specific IgE should be carefully confirmed, particularly in older children, by elimination and loading tests, as previously described. Moreover, the existence of food-specific IgE does not necessarily result in AD. Considerably large proportions of control infants without AD were positive for f1 RAST or f2 RAST even when the cut-off point was set at 0.70 Ua/mL. Because we did not examine the family history of atopic diseases in the control group, it is likely that infants with a family history of atopic disease existed in the control group. They may have increased the proportion of f1 RAST- and f2 RAST-positive results in control infants. Unfortunately, we could not follow up these infants in terms of the later development of AD. It is likely that food-specific IgE plays an important role in the development of AD in infants, but that the development of AD in infants with IgE against food involves other factors.

Although we understand the limited diagnostic specificity of IgE RAST for the detection of food allergy, we propose that IgE against egg white at a concentration of 0.15 Ua/mL or higher in infants with AD, particularly those younger than 6 months of age, has some diagnostic value for egg allergy for the following reasons. First, many AD infants without detectable f1 RAST later showed high f1 RAST levels. Moreover, although we did not confirm by elimination and loading tests that egg allergy was responsible for AD in the AD infants with low levels of IgE against egg white in the present study, elimination and loading tests in a previous study had shown that egg allergy was responsible for AD in more than 85% of AD infants with positive f1 RAST (≥ 0.35 Ua/mL), while this was the case in only 70% of AD children at 2–3 years of age (S. Ito, unpubl. obs., 1993). Furthermore, AD in children mainly develops within the first 6 months of life and the early detection of egg allergy followed by the early elimination of eggs from the diet may be useful in preventing the later development of allergy against other antigens. These data strongly suggest that low levels of IgE against f1 are useful, although not completely specific, for the early detection of egg allergy in AD infants. When the cut-off point was set at either 0.35 or 0.15 Ua/mL, the significant difference (P < 0.01) in the distribution of f1 RAST-positive and -negative subjects between atopic and non-atopic infants did not change. At the very least, f1 RAST should be examined repeatedly in AD infants with IgE against egg white at a concentration of ≥ 0.15 Ua/mL. Elimination and loading tests should be undertaken to further clarify the existence of egg allergy in AD infants with very low levels (0.15–0.35 Ua/mL) of egg white-specific IgE.

The proportion of infants with IgE against egg white increased after 6 months of age, even in non-atopic control infants. As Japanese infants usually start eating eggs after 5–6 months of age, the increase in the proportion of infants with IgE against egg white in the non-atopic control group was likely to be due to the increased egg intake. Because many infants in the present study who were fed cows’ milk were also fed breast milk and because we did not examine egg intake by the infants or their mothers, we could not determine whether there was a correlation between egg white-specific IgE levels and the levels of egg intake.

Interestingly, the proportion of AD infants with egg white-specific IgE was smaller in the group of infants older than 8 months of age compared with those at 6–8 months of age and f1-specific IgE levels decreased in 56% of AD infants at the time of re-examination within 1 year. It is suggested that the level of egg white-specific IgE starts decreasing at approximately 8 months of age in some AD infants. The early decrease of serum egg white-specific IgE levels in AD children has been noted previously.

The examination for IgE against cows’ milk showed similar results to those of IgE against egg white and f2 RAST at a concentration of ≥ 0.35 Ua/mL is considered to have a diagnostic value for milk allergy in infants. However, the very low level of IgE against cows’ milk (0.20–0.35 Ua/mL) was seen in non-atopic control infants more frequently than in AD infants. Therefore, the very low level of f2 RAST may be helpful for the diagnosis of milk allergy during the early infantile period, but is less reliable than f1 RAST.

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