Ovomucoid-specific IgD increases in children who naturally outgrow egg allergy

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Abstract

Background: The majority of egg-allergic children outgrow hypersensitivity against heated egg and then raw egg over time. The roles of ovomucoid (OVM)- and ovalbumin (OVA)-specific IgD in children who naturally outgrow egg allergy are uncertain. We investigated whether specific IgD to egg white (EW), OVM, and OVA correlate with allergen-specific IgE and can predict the development of immune tolerance to egg allergens. Methods: The tolerated doses of cooked egg white, which were determined by oral food challenge and/or an episode of accidental ingestion and corresponding specific IgE, IgG4 and IgD levels were evaluated in 57 children with egg allergy and 23 non-egg allergic children. Results: Patients avoiding all forms of egg had lower EW-, OVM-, and OVA-specific IgD and IgG4 than those partially avoiding egg, those that had outgrown egg allergy, and non-egg allergic children. The ratio of OVM-specific IgD to OVA-specific IgD increased depending on the ingestible amounts of boiled EW, whereas the ratio of OVM-specific IgG4 to OVA-specific IgG4 did not change. Receiver operating curve analysis revealed that the ratio of OVM-specific IgE to OVM-IgD was the best index to discriminate intolerant from tolerant egg-allergic patients. Conclusion: The production of OVM-specific IgD differs from OVM-specific IgG4 as children naturally outgrow egg allergy. The ratio of OVM-specific IgE to OVM-specific D is useful in distinguishing egg-sensitized patients with clinically reactive egg allergy from those who naturally outgrow egg allergy.

Original Article

Title: Ovomucoid-specific IgD increases in children who naturally outgrow egg allergy.

Short title: Ovomucoid-specific IgD in egg-allergic children

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egg white, food allergy, IgD, IgG4, ovomucoid

Conflict of interest:
The authors declare that they have no conflicts of interest.

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Author contribution:
NI, MY, AK, HM, EN, YH, and KO collected the samples. NI wrote the manuscript and YO organized the study. All authors read and approved the final manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (to YO).

Abstract

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Results: Patients avoiding all forms of egg had lower EW-, OVM-, and OVA-specific IgD and IgG4 than those partially avoiding egg, those that had outgrown egg allergy, and non-egg allergic children. The ratio of OVM-specific IgD to OVA-specific IgD increased depending on the ingestible amounts of boiled EW, whereas the ratio of OVM-specific IgG4 to OVA-specific IgG4 did not change. Receiver operating curve analysis revealed that the ratio of OVM-specific IgE to OVM-IgD was the best index to discriminate intolerant from tolerant egg-allergic patients.

Conclusion: The production of OVM-specific IgD differs from OVM-specific IgG4 as children naturally outgrow egg allergy. The ratio of OVM-specific IgE to OVM-specific D is useful in distinguishing egg-sensitized patients with clinically reactive egg allergy from those who naturally outgrow egg allergy.

1. Introduction

Several lines of evidence support the involvement of secreted IgD in mucosal immunity. Increased serum concentrations of total IgD and allergen-specific IgD have been reported in patients with allergic respiratory diseases. Interestingly, symptomatic atopic individuals exhibited higher serum IgD concentrations compared to asymptomatic atopic individuals. However, these findings are not consistent across studies. Beekeepers with tolerance to bee venom developed higher serum levels of IgD specific for the bee venom antigen, phospholipase A2, compared with healthy controls. Allergen-specific IgD-producing B cells expand during allergen-specific immunotherapy, and serum levels of allergen-specific IgD correlated with asthma control.

Furthermore, secreted IgD interacts with galectin-9, allowing IgD to bind to basophils and mast cells, which attenuates IgE-induced basophil degranulation. Together, these findings support the role of allergen-specific IgD in the development of allergen immune tolerance. However, ligation of basophil-bound IgD by antigen also induced basophil secretion of IL-4 and IL-13, which amplified T follicular helper type 2 cell-mediated
IgG1 and IgE production by B cells. Thus, the clinical significance of IgD in allergic disorders remains controversial.

Milk- and egg-allergic children treated with oral immunotherapy (OIT) mounted increased food antigen-specific IgD. Moreover, ovalbumin (OVA)-specific IgD increased in desensitized egg-allergic children but not in children unresponsive to OIT or with sustained unresponsiveness to OVA challenge. Although egg-allergic children had higher allergen-specific IgD levels compared to the atopic controls, egg allergic children with higher levels of allergen-specific IgD had a decreased risk of anaphylactic reactions. However, the potential role of IgD in the development of immune tolerance to food allergens remains to be elucidated.

The natural development of tolerance and the acquisition of sustained unresponsiveness by OIT in patients with egg allergy is associated with an increase in ovomucoid (OVM)-specific IgG4 levels and a decrease in OVM-specific IgE levels. In this study, we determined the relationship between egg white (EW)-, OVA-, and OVM-specific IgD, IgG4 and IgE in intolerant, partially tolerant, and tolerant egg-allergic children to elucidate the role of allergen-specific IgD in the outgrowing of egg allergy.

2. Material and methods

2.1. Study participants

Serum samples were collected from 57 egg-allergic and 23 healthy non-egg allergic (non-egg allergy: NEA) children and stored at -80°C until further analyzed. Egg-allergic children with EW-specific IgE levels greater than 0.70 kUA/L and apparent allergic reactions after ingestion of egg and egg products were enrolled in this study. The tolerated egg dose for each patient was determined by oral food challenge and/or an episode of accidental ingestion of cooked EW. The patients were classified into three groups: 28 patients avoided all forms of egg in the diet (complete avoidance of egg: CAE), 18 patients were able to ingest at least 1/32 cooked whole egg (low dose) but not one cooked whole egg (full dose) (partial avoidance of egg: PAE), and eleven patients outgrew egg allergy (OGE).

The study was approved by The Research Ethics Committee of University of Fukui (#20110052), and written informed consent was obtained from the parent or guardians.

2.2. Measurement of allergen-specific Ig levels

Serum levels of EW-, and OVM-specific IgE were measured using ImmunoCAP (Thermo-Fisher Inc., MA) according to the manufacturer’s instructions. Serum levels of EW-, OVM-, and OVA-specific IgD and IgG4 were determined by ELISA. In brief, microtiter plates (Nalgen Nunc International Co. Tokyo, Japan) were coated with 100 μL/well of EW, OVM, or OVA (100 μg/mL) overnight at 4°C and then were blocked by incubation with ELISA blocking reagent (Roche Diagnostics Co., Tokyo, Japan) for 3 hours at room temperature. After washing four times with PBS/0.05% Tween 20, the plates were incubated with 100 μL/well of diluted serum samples overnight at 4°C. After washing four times with PBS/0.05% Tween 20, plates were then incubated with 100 μL/well of biotin-conjugated rat anti-human IgD or IgG4 antibody (Southern Biotech, AL) at 2 μg/mL in PBS/0.05% Tween 20, followed by washing four times with PBS/0.05% Tween 20 and then a 2-hours incubation with 100 μL/well of horseradish peroxidase-conjugated streptavidin (X4000 in PBS/0.05% Tween 20, Thermo-Fisher Inc.). The plates were developed with the TMB microwell peroxidase substrate system (Kirkegaard and Perry Laboratories Inc., Guildford, UK). The antigen-specific antibody titers in the serum were expressed as arbitrary units determined using the standard pooled serum, which contained high titers of antigen-specific antibody.

2.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism ver 8.4.1(GraphPad Software, San Diego, CA). Kruskal-Wallis test and Dunn’s multiple comparison test were used to compare the antigen-specific Ig levels of each group. The correlation between antigen-specific IgE, IgD, and IgG4 levels was analyzed by Spearman’s rank correlation test. A p-value <0.05 was considered statistically significant.

3. Results
3.1. Subject characteristics

As shown in Table 1, subjects in the CAE, PAE, OGE, and NEA groups did not differ in age. However, the CAE group had a higher male to female ratio compared to the other groups. Patients with egg allergy, including those in the CAE, PAE, and OGE groups, tended to be more comorbid with other allergic diseases than the NEA group. The CAE and PAE groups had an increased family history of allergic diseases compared to the OGE and NEA groups. Total IgE, EW-specific IgE, and OVA-specific IgE levels were higher in the CAE group, followed by the PAE, OGE, and NEA groups (Table 1 and Fig 1).

3.2. Comparison of IgD and IgG4 serum levels in the egg allergic and NEA groups.

The CAE group exhibited lower serum levels of EW-specific IgD compared to the NEA group and lower serum levels of OVA-specific IgD compared to the PAE group (Fig. 1B and 1G). In addition, the CAE group had the lowest OVM-specific IgD serum levels among all of the groups (Fig. 1E). It has been reported that the natural development of tolerance by continuous ingestion of heated egg in egg-allergic patients is associated with an increase in EW-, OVM- and OVA-specific IgG4 levels. Consistent with these findings, we observed the lowest serum levels of EW-, OVA-, and OVM-specific IgG4 in the CAE group, followed by the PAE and OGE groups (Fig. 1C, F, and H).

OVM-specific IgD significantly correlated with OVM-specific IgG4 in all groups except the CAE group (Spearman’s rank correlation coefficient; \( \rho = 0.10, 0.78, 0.71, \) and \( 0.70 \) in CAE, PAE, OGE, and NEG groups, respectively). Furthermore, we observed a significant correlation between EW-specific IgD and IgG4 in the PAE group (\( \rho = 0.84 \)). These findings suggest that serum levels of OVM-specific IgD, but not EW-specific IgD, are associated with outgrowing egg allergy.

3.3. The relative levels of allergen-specific IgD but not IgG4 depend on the status of egg allergy

The relative levels of OVM-specific IgD were lower than those of OVA-specific IgD in the CAE groups (Fig 2A). In contrast, the relative levels of OVM-specific IgD were similar to levels of OVM-specific IgD in the PAE and OGE groups (Fig 2B and 2C). The relative levels of OVM-specific IgG4 and OVA-specific IgG4 did not differ between the groups (Fig. 2E-H). Therefore, the ratio of OVM-specific IgD to OVA-specific IgD increase as children outgrow egg allergy, whereas the ratio of OVM-specific IgG4 to OVA-specific IgG4 did not change (Fig 2I and 2J).

3.4. The ratio of OVM-specific IgE to OVM-IgD predicts outgrowing egg allergy.

The ratio of OVM-specific IgE to OVM-specific IgD or IgG4 in the CAE group was significantly higher compared to the PAE, OGE, and NEA groups (Figure 3). Receiver operating analysis revealed that the ratio of OVM-specific IgE to OVM-specific IgD discriminated non-tolerant from partially-tolerant egg-allergic patients with the largest area under the curve (AUC = 0.965) compared with levels of OVM-specific IgE or the ratio of OVM-specific IgE to OVM-specific IgG4 (Figure 4, Table 2). Thus, the ratio of OVM-specific IgE to OVM-specific IgD is the most useful marker to identify high-risk egg allergic patients. The optimal cutoff for the ratio of OVM-specific IgE to OVM-specific IgD had 86.5% sensitivity and 96.4% specificity to identify high-risk subjects (Table 2).

Discussion

In this study, we found that OVM-specific IgD serum levels increased in egg-allergic patients with increasing tolerable amounts of heated egg, which is consistent with previous reports of OVM-specific IgG4. As egg-allergic patients initially become able to ingest cooked egg during acquisition of natural tolerance, our findings suggest that OVM-specific IgD levels are associated with outgrowing egg allergy.

High-affinity, but not low-affinity, IgE is known to cause anaphylaxis. We hypothesized that elevated allergen-specific IgD levels are associated with low-affinity allergen-specific IgE levels as children outgrow egg allergy, resulting in hypo-responsiveness to egg allergens. High-affinity IgE antibodies are derived from...
memory B cells, whereas low-affinity IgE antibodies are derived from naïve B cells. A recent study demonstrated that allergen-specific IgG⁺ memory B cells prone to isotype switching without further affinity maturation are likely sources of short-lived IgE⁺ plasmablasts. The IgM and IgD constant regions are located in the first immunoglobulin heavy chain constant region gene block, whereas the IgG4 and IgE constant regions are located in the third gene block. During class switching from IgM to IgG or IgE, the IgD and IgM constant regions are removed through a looping-out deletion of the heavy chain DNA interposed between the IgM and IgG or IgE S regions. Naïve IgM⁺IgD⁺ B cells undergo IgM to IgD class switching and become IgD secreting IgD⁺ plasma cells. Therefore, allergen-specific IgD production might reflect low-affinity allergen-specific IgE production.

The ratio of OVM-specific IgD to OVA-specific IgD, but not that of OVM-specific IgG4 to OVA-specific IgG4, changed depending on the status of egg allergy. Studies show that IgE and IgG4 antibodies bind largely the same sequential epitopes in patients who remained reactive or outgrew their allergy. Overlapping binding patterns between IgE and IgG4 support the hypothesis that the differentiation of allergen-specific memory IgG⁺ B cells may shift from specific IgE producing cells to specific IgG4 producing cells during the development of allergen immune tolerance. As OVM-specific IgD production was preceded by OVA-specific IgD production, our study suggests that different mechanisms regulate allergen-specific IgD production compared to allergen-specific IgG4 production.

A previous study found that OVM-specific IgE is more effective at differentiating clinically reactive egg-allergic patients from those tolerant of heated egg compared to EW- and OVA-specific IgE, suggesting that reactivity to heated egg is dependent on the affinity of OVM-specific IgE. Recently, OVM-specific IgE avidity has shown to improve the predictive value for allergy and anaphylaxis to heated hen’s egg. OVM-specific IgD levels might reflect the replacement of high-affinity with low-affinity OVM-specific IgE and thereby discriminate intolerant from partially tolerant egg-allergic patients. On the other hand, even if relatively higher levels of OVA-specific IgD were accompanied with the presence of low-affinity of OVA-specific IgE in the CAE group, OVA-specific IgD could not predict outgrowing egg allergy.

Because of the largest AUC in ROC analysis, the ratio of OVM-specific IgE to OVM-specific IgD could be a useful marker to identify high-risk egg-allergic patients capable of ingesting a low-dose of cooked whole egg who might be a good candidate for low-dose OIT. In fact, low-dose OIT with 1/32 of a cooked whole egg has been shown to induce sustained unresponsiveness to 1/32 and 1/2 of a cooked whole egg, with no severe symptoms. Japanese food allergy guidelines recommend an oral food challenge test using a low cumulative dose, corresponding to 1/32 of a cooked whole egg in high-risk cases where a small dose may induce symptoms.

There are several limitations to this study. First, there was a small number of patients. Second, the sex ratio differed between groups; however, we do not believe that this influences our results as serum total IgD concentrations in adults are affected by age and smoking but not gender. Third, all children were only challenged with heated egg and were instructed to avoid egg of any form if they tested positive with less than 2g of boiled EW. Finally, there was a lack of trajectory of specific IgD levels during natural tolerance development. Further studies involving more patients are needed to elucidate the role of allergen-specific IgD in immune tolerance in egg-allergic patients.

In conclusion, OVM-specific IgD production differs from OVM-specific IgG4 as children natural outgrow egg allergy. The ratio of OVM-specific IgE to OVM-specific D is useful in distinguishing egg-sensitized patients with clinically reactive egg allergy from those partially tolerant to egg.

References


Table 1. Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>CAE (n=28)</th>
<th>PAE (n=18)</th>
<th>OGE (n=11)</th>
<th>NEA (n=23)</th>
<th>p-value</th>
</tr>
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<tr>
<td>Number</td>
<td>28</td>
<td>18</td>
<td>11</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>24 (85.7%)</td>
<td>9 (50%)</td>
<td>7 (63.6%)</td>
<td>10 (43.5%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>1 (1-2.25)</td>
<td>3 (2-4.5)</td>
<td>2 (1.5-3.5)</td>
<td>3 (1-4)</td>
<td>n.s.</td>
</tr>
<tr>
<td>AD (%)</td>
<td>18 (64.3%)</td>
<td>12 (66.7%)</td>
<td>7 (63.6%)</td>
<td>11 (47.8%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Family AD (%)</td>
<td>17 (60.7%)</td>
<td>12 (66.7%)</td>
<td>4 (36.4%)</td>
<td>7 (30.4)</td>
<td>0.0127</td>
</tr>
<tr>
<td>WBC (/μL)</td>
<td>11,200</td>
<td>7,350</td>
<td>8,500</td>
<td>8,600</td>
<td>n.s.</td>
</tr>
<tr>
<td>(8,300-14,800)</td>
<td></td>
<td>(6,300-8,925)</td>
<td>(7,100-9,800)</td>
<td>(7,500-11,300)</td>
<td></td>
</tr>
<tr>
<td>Eo (%)</td>
<td>3 (2-4.2)</td>
<td>3 (1.9-4.3)</td>
<td>2 (1.3-4)</td>
<td>2 (1-3)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plt (X10^4/μL)</td>
<td>34.7 (30.6-43.4)</td>
<td>20.2 (27.0-35.4)</td>
<td>35.4 (29.1-38.1)</td>
<td>33.7 (26.7-35.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total IgE (IU/mL)</td>
<td>158 (47.5-522.5)</td>
<td>140.5 (60.1-840)</td>
<td>22.3 (8.8-110.7)</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>(50.8-452.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EW-sIgE (UA/mL)</td>
<td>18.6 (2.9-35.5)</td>
<td>2.0 (1.1-6.2)</td>
<td>1.48 (0.98-2.59)</td>
<td>0.9 (0.3-1.4)</td>
<td>&lt;0.0001</td>
</tr>
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</table>
### Table 2

<table>
<thead>
<tr>
<th>parameters</th>
<th>AUC (95% CI)</th>
<th>Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
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<tbody>
<tr>
<td>EW-IgE</td>
<td>0.822 (0.732-0.911)</td>
<td>1.565</td>
<td>55.8</td>
<td>96.4</td>
<td>96.7</td>
<td>54.0</td>
</tr>
<tr>
<td>OVM-IgE</td>
<td>0.774 (0.667-0.881)</td>
<td>0.650</td>
<td>57.7</td>
<td>85.7</td>
<td>88.2</td>
<td>52.2</td>
</tr>
<tr>
<td>OVM-IgD</td>
<td>0.941 (0.876-1.000)</td>
<td>0.317</td>
<td>96.2</td>
<td>13.5</td>
<td>96.2</td>
<td>92.9</td>
</tr>
<tr>
<td>OVM-IgG4</td>
<td>0.864 (0.787-0.941)</td>
<td>0.094</td>
<td>69.2</td>
<td>96.4</td>
<td>97.3</td>
<td>62.8</td>
</tr>
<tr>
<td>OVM-IgE/IgD</td>
<td>0.965 (0.930-1.000)</td>
<td>0.468</td>
<td>86.5</td>
<td>96.4</td>
<td>97.8</td>
<td>79.4</td>
</tr>
<tr>
<td>OVM-IgE/IgG4</td>
<td>0.899 (0.831-0.968)</td>
<td>1.848</td>
<td>78.8</td>
<td>89.3</td>
<td>93.2</td>
<td>69.4</td>
</tr>
</tbody>
</table>

Optimal cutoffs were determined based on the Youden index, which is the distance between the point of inflection of the receiver operator characteristic curve and the reference line. Sensitivity, specificity, PPV, and NPV with 95% CIs are indicated for each cutoff.

NPV, Negative predictive value; PPV, positive predictive value.

### Figure legends

**Figure 1** Egg allergen-specific IgE, IgD, and IgG4 levels in egg-allergic and non-egg allergic patients (NEA). Egg white (EW)-, ovomucoid (OVM)- and ovalbumin (OVA)- specific IgE (A and D), IgD (B, E, and G), and IgG4 (G, F, and H) in the complete avoidance of egg (CAE), partial avoidance of egg (PAE), outgrown egg allergy (OGE), and NEA groups. *p<0.05, **p<0.01, ***p<0.001

**Figure 2** Relationship between ovalbumin (OVA)- and ovomucoid (OVM)-specific IgD or IgG4 in egg-allergic and non-egg allergic patients (NEA). Relationship between OVA- and OVM-specific IgD (A, B, C, and D) or IgG4 (E, F, G, and H) in the complete avoidance of egg (CAE) (A, and E), partial avoidance of egg (PAE) (B, and F), outgrown egg allergy (OGE) (C, and G), and NEA (D, and H) groups. The logarithms of the ratio of OVM-specific IgD to OVA-specific IgD (I) and that of OVM-specific IgG4 to OVA-specific IgG4 (J). *p<0.05, **p<0.01

**Figure 3** Relationship between ovomucoid-specific IgD, IgG4, and IgE in egg-allergic and non-egg allergic patients (NEA). The logarithms of the ratio of OVM-specific IgE to OVM-specific IgD (A), OVM-specific IgE to OVM-specific IgG4 (B), and OVM-specific IgD to OVM-specific IgG4 in the complete avoidance of egg (CAE), partial avoidance of egg (PAE), outgrown egg allergy (OGE), and NEA groups. *p<0.001
**Figure 4** Receiver-operating characteristic predicting tolerance to cooked egg white ingestion. Receiver-operating characteristic curves of OVM-specific IgE (long dashed double-dotted line), IgE/IgG4 (dotted line), IgD (broken line), and IgE/IgD (bold line).

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