

# Mapping of the IgE and IgG4 Sequential Epitopes of Ovomucoid with a Peptide Microarray Immunoassay

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## Key Words

Egg allergens • Epitope mapping • Ovomucoid • Peptide microarrays

## Abstract

**Background:** Ovomucoid (Gal d 1) has been demonstrated to be the most important allergen in IgE-mediated egg allergy. Peptide microarray analysis is a novel method that can provide useful information on the nature of specific allergens. **Methods:** A peptide microarray immunoassay was performed using a 15- and 20-amino acid (aa) library of overlapping peptides (3-offset) of the primary sequence of ovomucoid. Sera from 50 patients with IgE-mediated egg allergy and reactivity to ovomucoid, with more than 1 year of follow-up, and sera from 10 controls were tested. Peptides were considered major epitopes when the average weighted Z-score was greater than 3 and recognized by at least 20% of the patient's sera. Specific IgE epitopes were established on the basis of the IgE/IgG4 Z-score ratio. **Results:** The IgE and IgG4 recognition pattern was similar in both sets of peptides, but the signal intensity was generally higher in the 20-aa set. Thirty-four percent of the patients did not recognize any IgE

sequential peptide and 20% of the patients recognized more than 10 sequential peptides. We identified 3 major IgE B-cell epitopes in domains I and II of ovomucoid. IgE/IgG4 ratio analysis showed that peptides 1–2 (aa 4–20) and peptides 29–31 (aa 91–104) were specific IgE epitopes. **Conclusion:** By using peptide microarray immunoassay in egg-allergic patients, we established that 34% of the patients do not have any linear epitope recognized by IgE. Further studies are needed to determine the clinical relevance of this finding.

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## Introduction

Egg is one of the most common food allergens in childhood [1]. The estimated prevalence of clinical allergy to egg in children aged 1–5 years is 1.8% [2]. In most cases egg allergy is transient and most egg-allergic children become tolerant. Approximately half of patients become tolerant in 3 years and up to 66% in 5 years [3], and this occurs even though IgE antibodies to egg proteins persist in serum for several years [4, 5].

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Studies addressing the mechanisms behind egg allergy have shown that children with egg allergy have significant differences in molecular recognition of IgE-binding epitopes [6–9]. Egg white contains 24 different glycoproteins, and ovomucoid (OM) has been shown to be the most important and dominant allergen [10, 11]. OM belongs to the family of Kazal-type pancreatic secretory trypsin inhibitors and contains 186 amino acids (aa) forming 3 tandem domains designated DI, DII, and DIII [12]. OM has unique characteristics such as relative stability against heat [11] and proteinase digestion [13] and a strong allergenicity compared with other egg white components [10, 11]. These characteristics point to OM as a good candidate for studying the IgE-binding epitopes in egg food allergy.

The IgE-binding B-cell epitopes are usually classified as either linear or conformational based on the recognition of consecutive aa or the three-dimensional structure of the protein, respectively [14].

Different methods have been used for epitope mapping of food allergens: ELISA, Western blot, immuno-dot blot, radioallergosorbent test (RAST), SPOTs membrane, and microarrays. Among them, microarray-based immunoassay presents several advantages, such as: the possibility to assay thousands of targets simultaneously, the requirement of a low volume of serum, more robust statistical analysis, and the possibility to simultaneously test several immunoglobulin subclasses [15]. In the present study we used for the first time microarray technology to determine the sequential OM epitopes in sera of patients with egg allergy.

## Material and Methods

### Serum Samples

Children younger than 14 years, with a diagnosis of egg protein allergy were recruited from the Allergy Department of Hospital Ramón y Cajal from June 2007 to January 2009. The diagnosis of allergy to egg proteins was established on the basis of a suggestive clinical history and positive specific IgE (sIgE) to egg white, egg yolk, OM, and ovalbumin as determined by a skin prick test and/or by CAP System FEIA (Phadia, Uppsala, Sweden). The inclusion criteria were: (1) allergy to egg proteins with more than 1 year of follow-up, (2) clinical reactivity to egg proteins based on an immediate reaction to egg due to a diet transgression or a positive challenge test in the last 6 months of follow-up, and (3) levels of serum sIgE to OM higher than 3 kU/l at the time of inclusion into the study. The sera of 10 atopic and nonatopic healthy children with no history of food allergy were used as a negative control. The study protocol was approved by the Ethics Committee for Clinical Investigation of Hospital Ramón y Cajal.

### Microarray Printing

A library of overlapping peptides, with a length of 15 and 20 aa with an offset of 3 aa, corresponding to the primary sequences of OM, was commercially synthesized (GenScript Corporation, Piscataway, N.J., USA). Peptides were resuspended in PBS and diluted (1:1) with protein printing buffer (PPB; Arrayit Corporation, Sunnyvale, Calif., USA) to a final concentration of 1 mg/ml. All peptide samples were printed on glass slides coated with N-hydroxyl succinimidyl (NSB27 NHS; NanoSurface Biosciences-POSTECH, Seoul, Korea) using a SpotArray 72 spotter with SMP6 split pins (PerkinElmer, Waltham, Mass., USA). PBS/PPB (1:1) was used as a negative control and for background normalization.

### Microarray Immunolabeling and Detection

The slides were rinsed with PBS containing 0.1% Tween 20 (PBS-T), and nonspecific binding sites were blocked for 60 min with BlockIt™ blocking buffer (Arrayit Corporation) diluted 1:1 in PBS-T containing 2% BSA (Sigma-Aldrich, St. Louis, Mo., USA). The slides were washed twice with PBS-T, and patient sera diluted 1:2 in PBS-T 2% BSA were applied and incubated for 14 h on a rotator at 4°C. The slides were then washed twice with PBS-T and incubated for 2 h at room temperature with a mixture of monoclonal mouse anti-human IgE (clone G7-26) and IgG4 (clone G14-4) (BD-Pharmingen, San Diego, Calif., USA) diluted 1/1,000 in PBS-T 2% BSA (working concentration, 0.4 mg/ml), which had been covalently tagged with fluorochromes Alexa® 546 and Alexa 647. Finally, the slides were washed twice with PBS-T, dried, and scanned with a ScanArray Express (PerkinElmer, Waltham, Mass., USA).

### Data Analysis

Microarray analysis was performed according to the method of Lin et al. [15, 16]. This method is based on the calculation of a Z-score, which is a measure of the standardized fluorescent intensity for IgE and IgG4 recognition. In brief, the Z-score was calculated for each peptide spot ( $Z_i$ ) using the PPB values within the same array according to the formula:  $Z_i = (S_i - S_{PPB}) / \text{MAD}(S_{PPB})$ , where  $S$  for each peptide spot ( $S_i$ ) or PPB ( $S_{PPB}$ ) is the median fluorescence signal of the spot divided by local background and  $\log_2$  transformed.  $\text{MAD}(S_{PPB})$  is the median absolute deviation of all the read outs of PPB spots. The total Z-value for each peptide is the median of the Z-scores of the 3 replicate spots within the same microarray and denotes the average of standardized intensities of fluorescence (ch1 IgG4-Alexa 647 and ch2 IgE-Alexa 546). Finally, because of peptide overlapping, weighted averages of Z-values were calculated according to the formula:  $Z = 0.25 \cdot Z_{-1} + 0.5 \cdot Z_1 + 0.25 \cdot Z_{+1}$ . An individual peptide sample was considered positive if the weighted average Z-score exceeded 3 after subtracting the average Z-score controls for each peptide. Peptide 51, in its 15-aa and 20-aa series, was excluded from analysis because of its non-specific binding. Data analysis and presentation were performed using Microsoft Excel, Sigmaplot (Systat Software, Inc., San Jose, Calif., USA), and TIGR MultiExperiment Viewer (Mev v3.1) software. For the statistical analysis of epitope recognition, we performed the one-sample Student t test to reject the null hypothesis that the ratio of IgE to IgG4 standardized binding intensities equals zero. All of these tests were done with Mev v3.1 software. We did not use any false discovery correction, and  $p < 0.05$  was considered statistically significant. Peptides were considered ma-

major epitopes if the average weighted Z-score was greater than 3 and, additionally, if they were recognized by at least 20% of the patient's sera. Major sIgE epitopes were identified based on the IgE/IgG4 Z-score ratio, when the ratio was >1 in more than 20% of the patient's sera.

## Results

### *Characteristics of the Patients*

Fifty egg-allergic patients were included in the study. At the time of inclusion into the study, the clinical reactivity to egg was established by a positive oral challenge test in 31 patients; in another 8 patients we did not perform an oral challenge test because of a clinical history of anaphylaxis. Additionally, 9 patients referred positive egg transgressions within the 6 months prior to inclusion into the study and 2 patients rejected the egg oral challenge tests. The clinical characteristics of the patients are shown in table 1. The age of the patients ranged between 3 and 14 years, with a median age of 4 years and 5 months; 68% of children were male, and the most frequent symptom was urticaria. Severity criteria were defined, by consensus among all of the investigators, as mild (localized erythema or urticaria), moderate (generalized urticaria and/or angioedema, digestive symptoms, rhinitis, and cough), and severe (severe bronchospasm and/or breathing difficulties with inspiratory stridor, hypotension, and anaphylaxis), with percentages of 24, 62, and 14%, respectively. The median follow-up from diagnosis was 2 years and 8 months (1–13 years).

### *IgE and IgG4 Antibody Binding to Sequential Epitopes of OM*

We produced a microarray containing 15-aa and 20-aa overlapping peptides (offset of 3 aa) corresponding to linear sequences of OM. IgE and IgG4 sequential peptide recognition was detected simultaneously using 2 distinct fluorescence channels. Firstly, we assessed the quality of the microarrays by checking the signal-to-noise ratio (SNR) for IgE and IgG4 for each peptide, both in nonallergic controls and allergic patients. As shown in figure 1, IgE and IgG4 binding to OM was greater in sera from allergic patients than in those from controls. Using an arbitrary cutoff of log SNR <0.5, the percentage of 15-aa peptides recognized by IgE was 2.77% in allergic patients and none in controls. On the other hand, the percentage of 20-aa peptides recognized by IgE was 4.89% in allergic patients and 1.75% in controls. Similar results were obtained for IgG4 recognition (3.19% in allergic patients

**Table 1.** Clinical characteristics of patients (n = 50)

|                                                         |                   |
|---------------------------------------------------------|-------------------|
| Median age                                              | 4 years 5 months  |
| Range, years                                            | 3–14              |
| Male sex                                                | 68%               |
| Clinical symptoms, n                                    |                   |
| Cutaneous                                               | 29                |
| Digestive                                               | 8                 |
| Respiratory                                             | 1                 |
| Cutaneous + digestive                                   | 6                 |
| Cutaneous + respiratory                                 | 3                 |
| Cutaneous + digestive + respiratory                     | 3                 |
| Median skin prick test results (range), mm              |                   |
| Egg white                                               | 11 (5–24)         |
| Yolk                                                    | 10 (4–24)         |
| Ovalbumin                                               | 13 (5–26)         |
| OM                                                      | 14 (3–30)         |
| Median in vitro test results (25th to 75th percentiles) |                   |
| Total IgE, kU/l                                         | 345 (81.5–607)    |
| Egg white sIgE, kAU/l                                   | 11.8 (6.26–38)    |
| Yolk sIgE                                               | 2.4 (1.04–7.73)   |
| OA sIgE                                                 | 11.6 (6.24–27.62) |
| OM sIgE                                                 | 11.25 (5.7–18.22) |

Cutaneous symptoms are: urticaria and/or angioedema and/or atopic dermatitis. Digestive symptoms are: vomiting and/or diarrhea and/or abdominal pain. Respiratory symptoms are: rhinitis and/or cough and/or dyspnea and/or wheezing.

and 1.05% in controls for 15-aa peptides; 4.6% in allergic patients and 1.43% in controls for 20-aa peptides). Comparing the SNR obtained for 15 and 20 aa, peptides with the higher length gave a higher signal intensity for both IgE and IgG4.

For epitope binding quantification, we calculated the Z-score according to the methods described by Lin et al. [15, 16]. In figure 2 we show the weighted Z-score obtained for each peptide in control and allergic samples for both IgE and IgG4 fluorescence. As expected, the signal is associated with sets of overlapping peptides, indicating recognition of specific sequences. The percentage of IgE-positive peptides in allergic patients (average Z-score >3) was 11.37% (324 of 2,850) for the set of 20 aa and 8.21% (238 of 2,900) for the set of 15 aa. The percentage of positive IgG4 peptides in allergic patients was 8.84% (252 of 2,850) for the set of 20 aa and 8.17% (237 of 2,900) for the set of 15 aa. Sixty-six percent of the patient's sera were positive for at least one peptide recognized by IgE in the set of 20 aa and 56% in the set of 15 aa. For IgG4 the percentage of recognition of at least one peptide was 52 and 68%, respectively. The average proportion of peptides

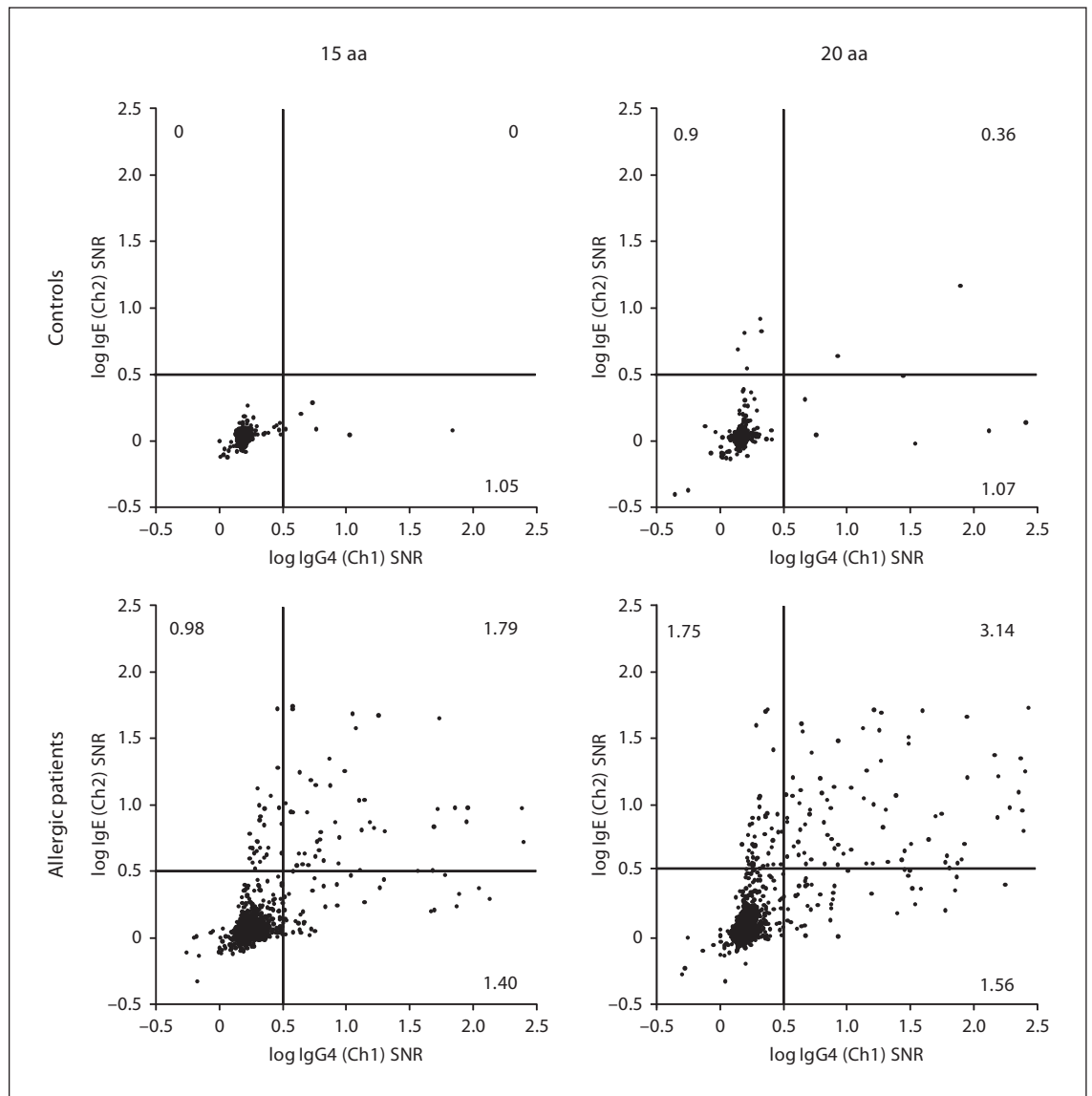


**Fig. 1.** Scatter plot of IgE and IgG4 fluorescence expressed as the SNR on microarray elements for 15-aa and 20-aa peptides of OM in sera from control subjects ( $n = 10$ ) and egg-allergic patients ( $n = 50$ ). Numbers indicate percentages above the cutoff ( $\log \text{SNR} > 0.5$ ).

recognized by IgE for each patient was 6.48% (range 0–41) for the set of 20 aa and 4.75% (range 0–34) for the set of 15 aa. Overall, the recognition pattern for IgE and IgG4 was similar for both 15- and 20-aa peptide sets, although with a higher signal intensity for the 20-aa peptides (fig. 2). Therefore, in order to determine the IgE-specific epitopes the set of 20 aa peptides was used.

Hierarchical cluster analysis of the average Z-score for IgE recognition showed a continuous distribution of the number of peptides recognized by the patient's sera, from

sera that do not recognize any peptide to those with high recognition (up to 41 peptides) (fig. 3). Sixty-six percent of patient's sera recognized at least one peptide and 34% of them did not recognize any peptide. The percentile distribution of IgE peptide recognition was: 25th percentile, 0 peptides; 50th percentile (median), 2 peptides, and 75th percentile, 10 peptides.

We found no correlation between a greater number of peptides recognized in microarray immunoassay and a longer duration or a greater severity of the disease ( $p =$

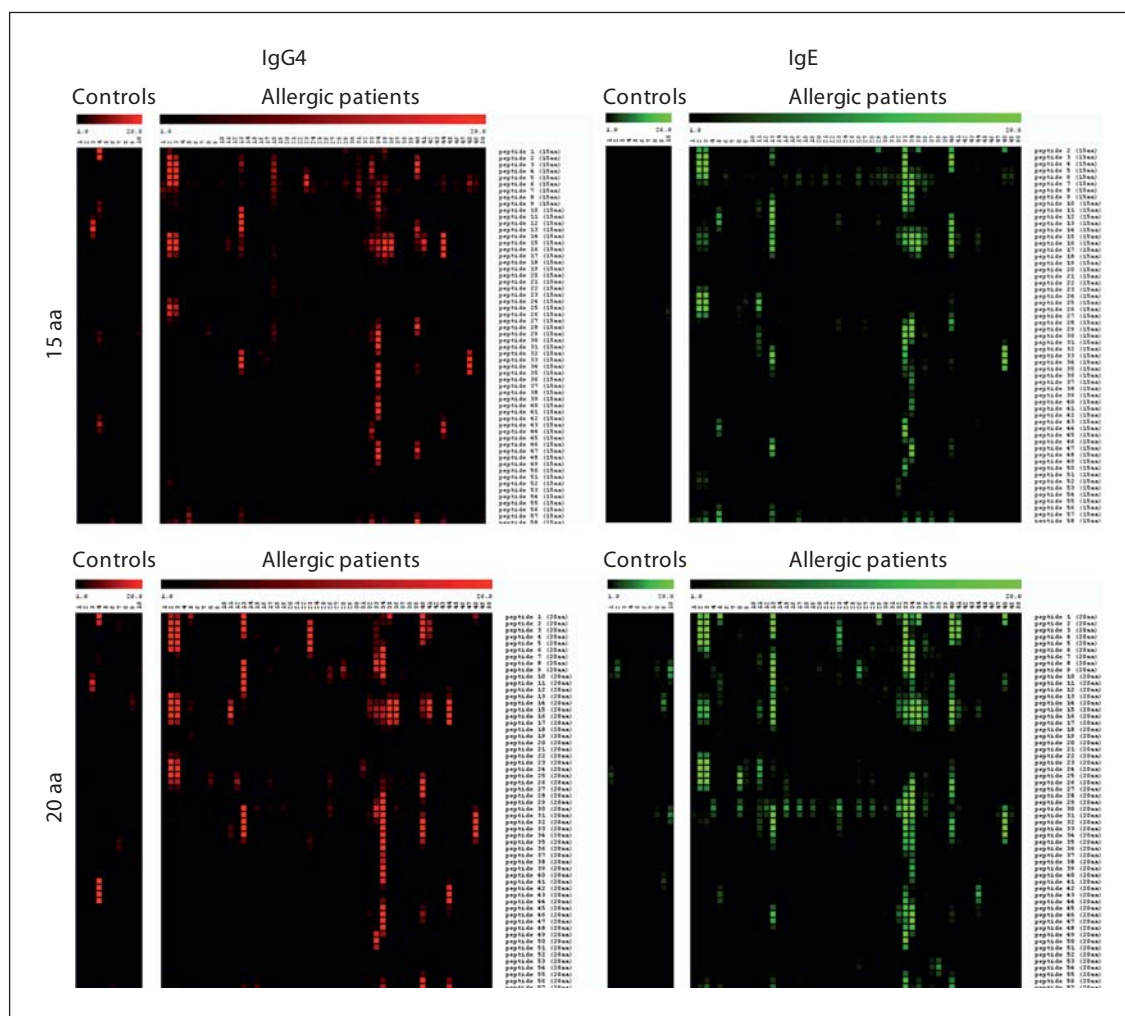


**Fig. 2.** IgE and IgG4 peptide binding to overlapping peptides corresponding to a lineal sequence of OM. The intensity of IgG4 and IgE binding is represented by weighted average Z-scores on a grading scale according to the scale bar at the top of the figure. Individual sera of control subjects and allergic patients are in columns, and 15-aa and 20-aa peptide sets are in rows.

0.090 and  $p = 0.097$ , respectively). When comparing peptide recognition with daily clinic diagnostic methods, there was a moderate but statistically significant association between the number of peptides recognized and the following variables: serum sIgE levels to egg white (Spearman's  $\rho = 0.479$ ,  $p < 0.001$ ) and serum sIgE levels to OM (Spearman's  $\rho = 0.450$ ,  $p < 0.001$ ).

When the percentage of patients with positive recognition for each peptide was analyzed, 3 major IgE recognition regions or epitopes were found: peptides 1–2 (aa 4–20), peptides 14–16 (aa 46–59), and peptides 29–31 (aa 91–104) (fig. 3). The first epitope was recognized by 26% of the patient's sera, the second by 24%, and the third by

38%. It is also important to mention that there were 3 regions that almost completely lacked IgE recognition: peptides 19–21 (aa 61–74), peptides 38–41 (aa 121–131), and peptides 50–52 (aa 154–167).

#### *sIgE Binding to Sequential Epitopes of OM*

It has been previously reported that the allergen-sIgE/IgG ratio could be associated with reactivity or tolerance to a specific food allergen [17–19]. To identify the predominant IgE epitopes we calculated the IgE/IgG4 Z-score ratio, whose hierarchical cluster distribution is shown in figure 4. Overall, 10 peptides presented a ratio of IgE to IgG4 which was significantly greater than zero

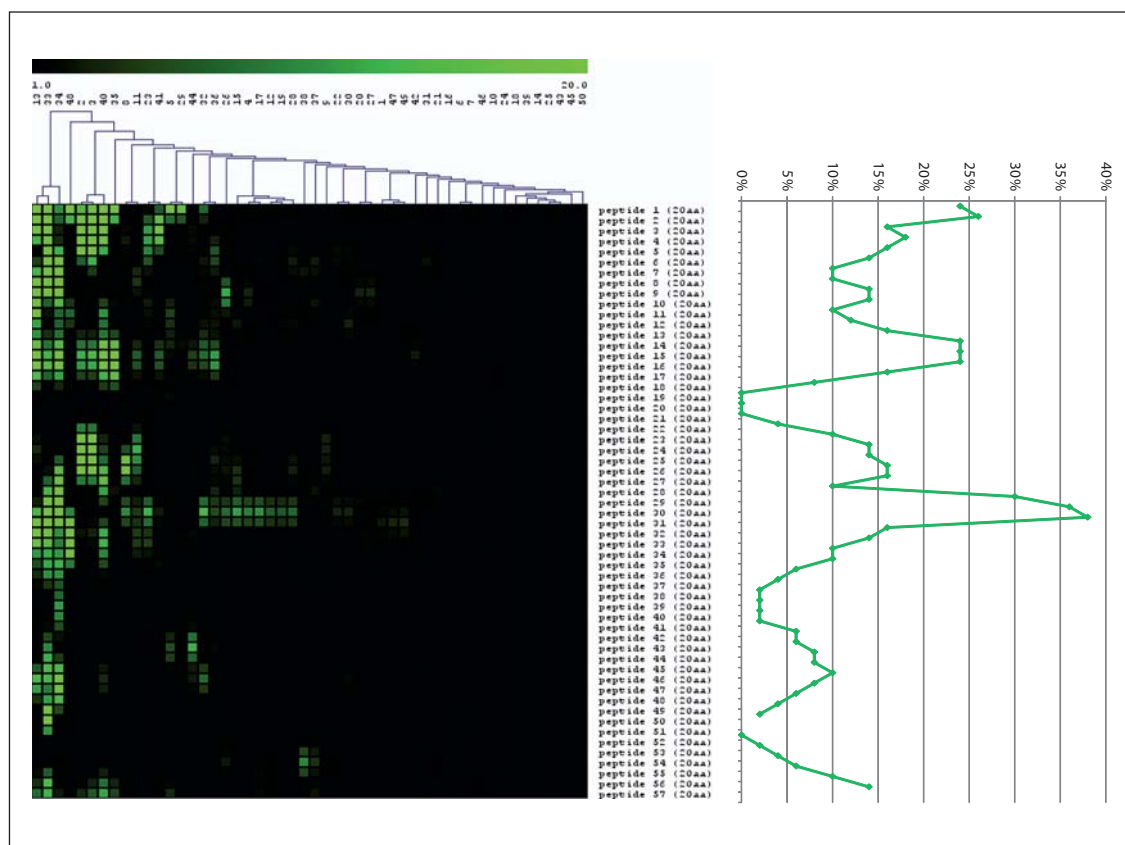


**Fig. 3.** Cluster analysis of IgE signal and percentage of patients showing positive binding to each peptide. The intensity of IgE binding is represented by weighted average Z-scores. Increasing green intensity denotes increased IgE binding according to the scale bar at the top of the figure. Allergic patients are in columns, and 20-aa peptides are in rows.

$[\log_{10} (Z \text{ IgE}/Z \text{ IgG4}) > 0]$ , and were considered IgE-binding peptides (fig. 4). Among them, 5 peptides were recognized by at least 20% of the patient's sera: peptides 1 and 2 in Kazal-like domain I and peptides 29, 30, and 31 in Kazal-like domain II (fig. 5). In conclusion, using microarray epitope mapping technology we identified 2 major sIgE epitopes in our egg-allergic population: epitope 1 (AEVDCSRFPNATDKEGKD), recognized by 24% of the patient's sera, and epitope 2 (VLCNRAFNPVCGT-DGVTYDNECLLCA), recognized by 32% of the patient's sera. Seven (14%) patients recognized both epitopes.

## Discussion

IgE-mediated egg allergy is a common childhood food allergy and it was demonstrated that OM is the most important egg allergen. Monitoring egg sIgE levels is useful

in the prognosis of the allergic disease [20]. In addition, high ratios of IgG4/IgE antibodies to food allergens are associated with a faster achievement of clinical tolerance [18].

In the last few years, the IgE-binding epitopes of different food allergens have been identified. Although food allergens contain both conformational and sequential epitopes, the importance of sequential epitopes in food allergy has been established. Sensitization to sequential epitopes has been associated with allergy to milk, peanuts, and eggs and with a different clinical pattern [7, 21, 22].

Most of the data regarding the sequential OM IgE epitopes have been obtained through the use of SPOT membrane-based immunoassays [7, 23]. The development of peptide microarray technology has stimulated research on the specificity and diversity of the IgE response to allergens [24]. In the present work we developed for the first time a peptide microarray for IgE and IgG4 epitope mapping in patients with egg allergy.

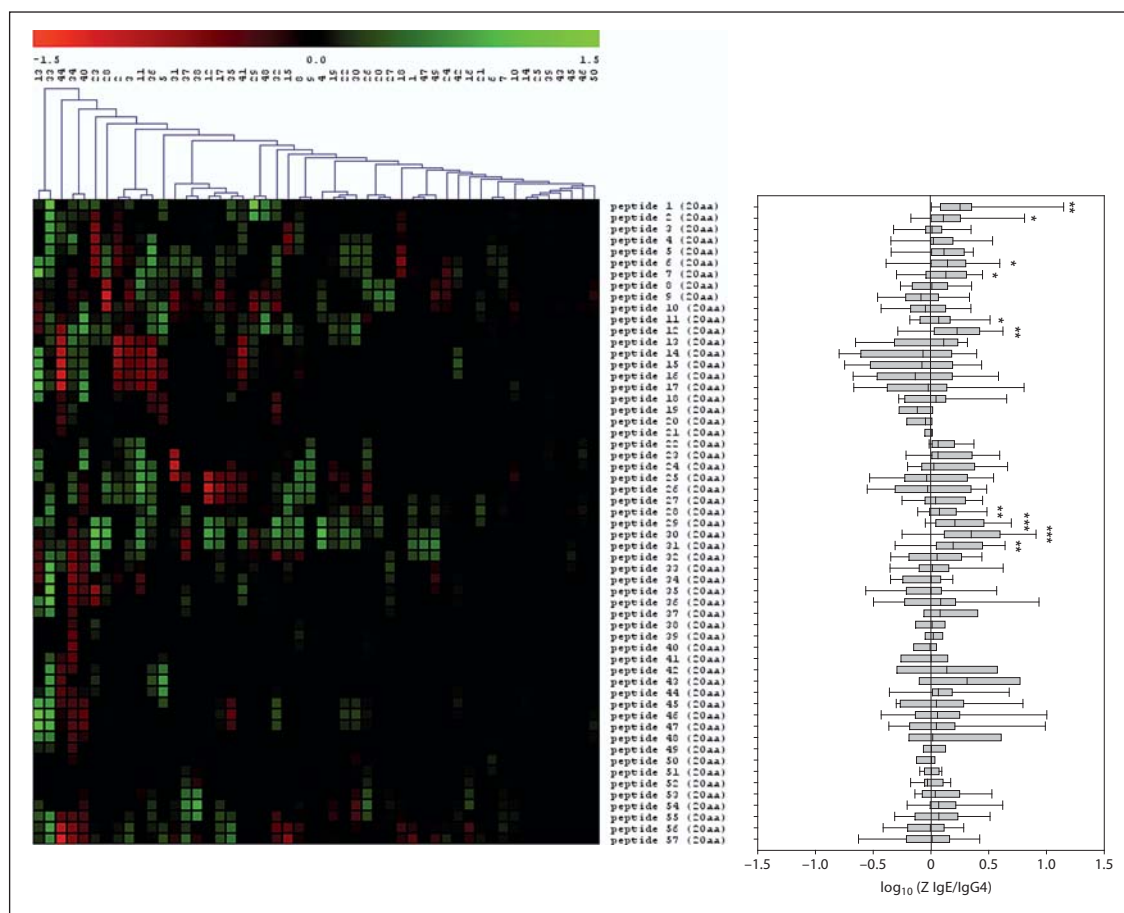


**Fig. 4.** Hierarchical cluster distribution of the IgE/IgG4 Z-score ratio. Increasing green intensity denotes predominant IgE recognition (IgE/IgG4 ratio  $>1$ ) and increasing red intensity denotes predominant IgG4 recognition (IgE/IgG4 ratio  $<1$ ) according to the scale bar at the top of the figure. Allergic patients are in columns, and 20-aa peptides are in rows. The box-and-whisker plot

represents the  $\log_{10}$  of the IgE/IgG4 Z-score ratio for each peptide. The boxes represent the medians and interquartile ranges. Whiskers represent the 10th and 90th percentiles. For statistical analysis we performed the one-sample Student t test to reject the null hypothesis that  $\log_{10}$  of the IgE/IgG4 Z-score ratio equals zero. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

The first goal of this study was to determine the best length of peptides for IgG4 and IgE recognition. The results of this study demonstrate greater efficacy in identifying allergenic peptides using a peptide length of 20 aa as compared with a peptide length of 15 aa. Previously, only one study had addressed this question. Shreffler et al. [25] compared 10-, 15-, and 20-aa peptides of Ara h 2, a peanut allergenic protein, and observed that signal intensity for both IgE and IgG4 increased as a function of peptide length. To explain this phenomenon, they suggested that longer peptides have higher affinity interactions or present multiple epitopes. Our results confirm that 20-aa peptides are the appropriate length to study allergenic peptides by microarray technology, for both IgE and IgG4 recognition.

In vitro studies on OM allergenicity have shown that fragments of this protein have a special stability against heat and digestion with proteases. In fact, some of the OM proteolytic fragments produced by peptic digestion retain their capacity to induce an allergic IgE response [8, 26]. In light of these studies it has been suggested that antibodies to OM mainly recognize sequential antigenic determinants. In contrast, clinical studies have determined that about 50% [11, 20] to 70% [27, 28] of egg-allergic patients tolerate heated egg, thus suggesting the predominance of IgE against conformational epitopes, instead of linear epitopes.

According to the IgE recognition of OM peptides, the present results allow us to establish two different groups

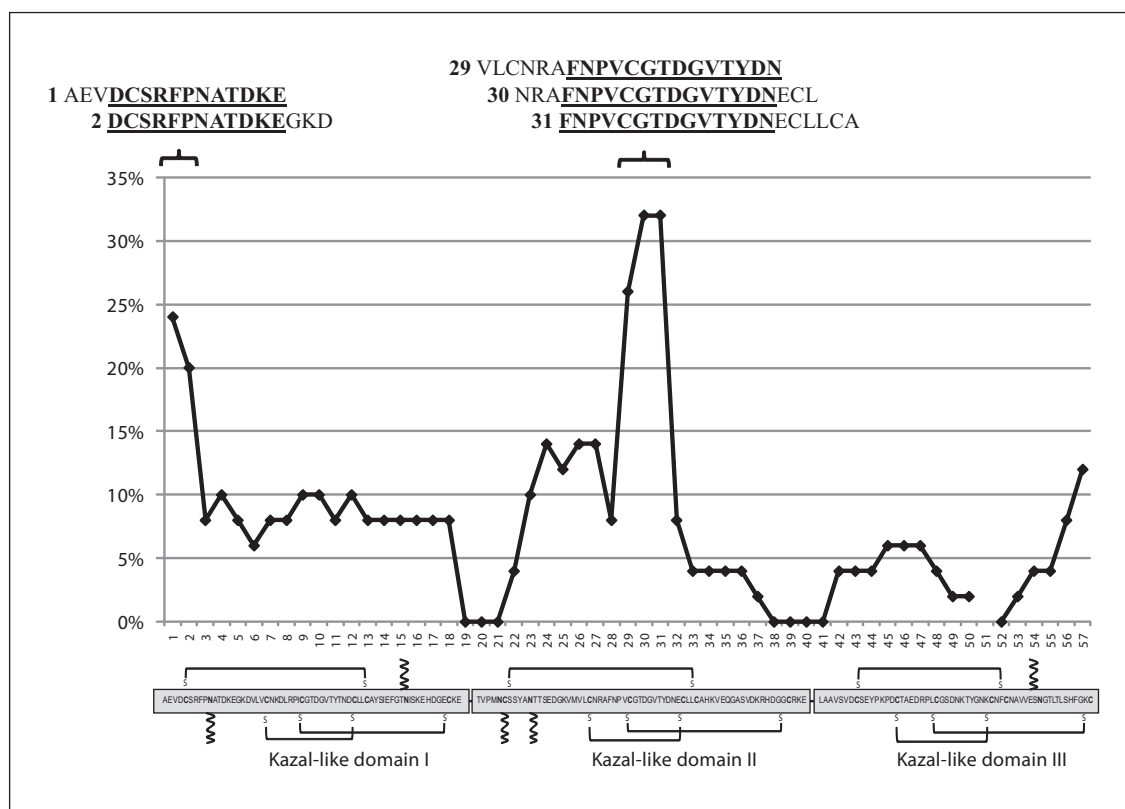


**Fig. 5.** sIgE epitope identification in OM. The x-axis shows the overlapping peptides according to the primary sequence of OM and the structural representation of the protein (Kazal-like domains, disulfide bonds, and N-glycan chains). The y-axis shows the percentage of patients with predominant IgE recognition (IgE/IgG4 Z-score ratio >1) for each peptide. The sequence of the major epitopes is seen at the top of the figure.

in the egg-allergic population studied herein: 66% of the patients recognized at least one OM peptide and 34% did not recognize any OM sequential epitope. According to these results, at least one third of the patients allergic to OM had reactivity to only conformational epitopes. On the other hand, 20% of the patients recognized more than 10 OM peptides (75th percentile), which may indicate a high recognition of sequential epitopes in such patients. Similar results were observed with other food allergens such as shrimp and lentil, where some of the allergic patients did not show significant binding to any shrimp peptide or Len C 1, respectively [29, 30].

This was a pilot, cross-sectional study in egg-allergic patients, designed to set up a peptide recognition technique using microarrays. The absence of a statistically significant correlation between the immunophenotypes of peptide recognition and clinical data may be due to the cross-sectional design of the present study. However, given the significant correlation between microarray pep-

tide immunophenotypes and the measurement of serum sIgE, as found herein, our technique is a promising tool for the characterization of allergic patients.

Most of the previously known OM epitopes for IgE and IgG have been identified by a SPOT array technique, ELISA, or Western blot (table 2). In the present study we identified 3 major IgE B-cell epitopes: peptides 1–2 (aa 4–20), peptides 14–16 (aa 46–59), and peptides 29–31 (aa 91–104). Among them, aa 4–20 and aa 91–104 present sIgE recognition epitopes according to the IgE/IgG4 Z-score ratio. Comparing the present results with earlier published data (table 2), all three IgE B-cell epitopes described herein using microarray technology are coincident with those previously identified using the above mentioned techniques, although some differences between studies can be found in the number and location. These differences could be attributed to the different technology used, the nature and length of the peptides, the sample used, and the population studied.

**Table 2.** Review of IgE-binding epitopes of OM (Gal d 1)

|                          | Cooke and Sampson [6]   | Besler et al. [32]                               | Holen et al. [31]                                       | Mine and Wei Zhang [23]                                        | Järvinen et al. [7]                                                   | Martínez-Botas et al. [present study]         |
|--------------------------|-------------------------|--------------------------------------------------|---------------------------------------------------------|----------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------|
| Analysis                 | SPOT array              | Western blot                                     | ELISA                                                   | SPOT array                                                     | SPOT array                                                            | fluorescent microarray                        |
| Samples                  | pooled sera             | pooled sera                                      | individual sera                                         | pooled sera                                                    | individual sera                                                       | individual sera                               |
| Peptides                 | 12 aa<br>offset by 2 aa | from chemical and<br>enzymatic cleavage<br>of OM | 14 aa<br>overlapping<br>by 4 aa                         | 10–15 aa<br>offset by 1 aa                                     | 10 aa<br>offset by 2 aa                                               | 15 and 20 aa<br>offset by 3 aa                |
| Domain I<br>OM 1–64      | OM 1–20<br>OM 49–50     |                                                  | OM 1–14<br>OM 11–24<br>OM 31–44<br>OM 51–64<br>OM 61–74 | OM 32–42<br>OM 40–50<br>OM 56–66                               | OM 1–10 <sup>a</sup><br>OM 9–20 <sup>a</sup><br>OM 47–56 <sup>a</sup> | OM 4–20 <sup>b</sup><br>OM 46–59 <sup>c</sup> |
| Domain II<br>OM 65–130   | OM 85–96<br>OM 115–122  | OM 90–121                                        | OM 101–114<br>OM 121–134                                | OM 71–75<br>OM 80–90<br>OM 101–105<br>OM 121–130<br>OM 159–174 | OM 113–124 <sup>a</sup>                                               | OM 91–104 <sup>b</sup>                        |
| Domain III<br>OM 131–186 | OM 175–186              | OM 134–186                                       |                                                         | OM 179–186                                                     |                                                                       |                                               |

<sup>a</sup> Denoted as major binding sites; <sup>b</sup> IgE specific recognition; <sup>c</sup> IgE and IgG4 recognition.

Interestingly, 3 regions of OM almost completely lacked IgE recognition: peptides 19–21 (aa 61–74), peptides 38–41 (aa 121–131), and peptides 50–52 (aa 154–167). The first 2 regions were located at the junctions between the 3 Kazal-like domains, where a special folding may impair the accessibility to generate antibodies against those regions. However, other explanations cannot be excluded given that previous studies have reported epitopes in such regions [23, 31, 32]. The third region with low IgE recognition was located in the third Kazal-like domain. It has been reported that N-glycans in the third domain are essential for the allergenicity of this domain [33], which can explain the small number of sequential epitopes found herein. However, a more recent report suggested that the carbohydrate chains in this domain are not necessary for OM allergenicity [8].

In summary, in the present work we used for the first time microarray technology to determine the immunophenotype of patients with egg allergy. We found that only a small number of patients (about one fifth) recognized a high number of sequential epitopes. By contrast, 34% of the patients did not recognize any sequential epitope. We also found 2 major immunogenic regions recognized by more than 20% of the egg-allergic patients. A

longitudinal study is needed to establish the true clinical relevance of the distribution of conformational and sequential epitopes, including the major epitopes found herein. Consequently, microarray-based IgE/IgG4 epitope mapping may become a useful tool for egg allergy diagnosis and prognosis.

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