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Epitope analysis of Ara h 2 and Ara h 6: characteristic patterns of IgE-binding fingerprints among individuals with similar clinical histories

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Summary

Background Ara h 2 and Ara h 6 are moderately homologous and highly potent peanut allergens.

Objective To identify IgE-binding linear epitopes of Ara h 6, compare them to those of Ara h 2, and to stratify binding based on clinical histories.

Methods Thirty highly peanut-allergic subjects were stratified by clinical history. Sera were diluted to contain the same amount of anti-peanut IgE. IgE binding to overlapping 20-mer peptides of Ara h 2 and Ara h 6 was assessed using microarrays.

Results Each subject had a unique IgE-binding fingerprint to peptides; these data were coalesced into epitope binding. IgE from subjects with a history of more severe reactions ($n = 19$) had a smaller frequency of binding events (BEs) for both Ara h 2 (52 BEs of 152 (19X8 epitopes) possible BEs) and Ara h 6 (13 BEs of 133 (19X7 epitopes) possible BEs) compared to IgE from those with milder histories ($n = 11$) (Ara h 2: 47 BEs of 88 (11X8 epitopes) possible BEs, $P < 0.01$; Ara h 6: 25 BEs of 77 (11X7 epitopes) possible BEs, $P < 0.001$). Using an unsupervised hierarchical cluster analysis, subjects with similar histories tended to cluster. We have tentatively identified a high-risk pattern of binding to peptides of Ara h 2 and Ara h 6, predominantly in subjects with a history of more severe reactions (OR = 12.6; 95% CI: 2.0–79.5; $P < 0.01$).

Conclusions and Clinical Relevance IgE from patients with more severe clinical histories recognize fewer linear epitopes of Ara h 2 and Ara h 6 than do subjects with milder reactions and bind these epitopes in characteristic patterns. Close examination of IgE binding to epitopes of Ara h 2 and Ara h 6 may have prognostic value.

Keywords allergens, Ara h 2, Ara h 6, IgE, microarrays, peanut, peptides

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Introduction

Peanuts are a major cause of severe IgE-mediated food allergy, which affects approximately 1–2% of the population in the United States [1, 2]. Allergy to peanuts rarely resolves and persists in approximately 80% of peanut-allergic patients [3]. Levels of anti-peanut IgE > 14 kU/mL and skin tests of > 5 mm are associated with a positive oral peanut challenge, while binding of IgE to Ara h 2 is associated with clinical reactivity to peanuts. However, there are no *in vitro* tests that correlate well with the severity of clinical history or responses to food challenges [4–8].

As of 2014, thirteen peanut allergens (Ara h 1–13) have been officially recognized by the International

Union of Immunological Societies, Allergen Nomenclature Sub-Committee (<http://www.allergen.org/search.php?allergen=peanut&searchsource=Search>). Of these, two 2S albumins, Ara h 2 and Ara h 6, together account for approximately 10% of the peanut proteome and are recognized by IgE from most peanut-allergic children and adults [9, 10]. Ara h 2 and Ara h 6 are moderately homologous in both their primary and secondary structure (4 and 5 disulphide bonds, respectively), are the most potent peanut allergens, and are substantially redundant in their biologic activity [11–21].

Microarray immunoassays with overlapping peptides that bind IgE have been described for a variety of food allergens. The potential utility of these assays has recently been reviewed [6, 22–36]. Peptide-based

microarrays have been used to distinguish truly peanut-allergic subjects from those who are sensitized but not clinically allergic [37].

Patients with a more severe clinical history have been reported to have IgE that recognizes a high number of linear epitopes of Ara h 1, 2, and 3 [29, 38]. In support of these findings, IgE from milk-allergic subjects with a higher grade of anaphylaxis following allergen challenge bound a greater number of milk peptides than did IgE from other milk-allergic subjects [32]. In contrast, other studies have demonstrated that epitope diversity correlates better with levels of food-specific IgE than with clinical history. For example, Ayuso *et al.* found that young age and higher shrimp-specific IgE, but not clinical history, was associated with more epitope diversity for shrimp allergen peptides [31]. Verada *et al.* [39] reported that epitope recognition for lentil allergen peptides, as well as respiratory symptoms, were associated with IgE levels, but did not find an association between epitope recognition and respiratory symptoms independent of IgE levels. Willumsen and colleagues reported that the complexity of the Der p 2-specific IgE repertoire increases with the concentration of Der p 2-specific IgE [40]. Thus, levels of allergen-specific IgE can be an important factor in interpretation of peptide-based microarray assays of IgE binding.

Given the potent effector activity and structural homology of Ara h 2 and Ara h 6, we have developed a microarray with Ara h 2 and Ara h 6 peptides to examine IgE binding to linear epitopes of these proteins. This assay, in which we normalize each serum to equivalent concentrations of peanut-specific IgE to remove the level of peanut-specific IgE as a confounding variable, demonstrates subtleties regarding IgE binding to linear epitopes of these two related proteins and an unexpected correspondence of these findings to the severity of clinical histories. This approach may have clinical utility in identifying subjects at risk for more severe clinical reactions.

Materials and methods

Subjects and their classification

We enrolled 47 subjects, ages 7–70 years, with a strong history of peanut-induced immediate hypersensitivity. These individuals were referred by local allergists, responded to university-wide emails, volunteered at a booth set-up at The Walk for Food Allergy, or were referred by other subjects. We also enrolled one subject with the hyper-IgE and recurrent infection syndrome (HIE) with a total IgE of 19 736 IU/mL. This serum was diluted 1 : 5, had undetectable anti-peanut IgE, and served as an IgE control. Of these 47 subjects enrolled, we identified 30 peanut-allergic subjects who met the fol-

lowing criteria: (i) physician-diagnosed peanut allergy and (ii) peanut-specific IgE ≥ 14 KAU/L (ImmunoCap, Phadia; Uppsala, Sweden) in serum collected within 6 months of this study, a finding that is consistent with 95% confidence of true peanut allergy [5]. Sera from these 30 subjects were used in these studies. These subjects, who were rigorously avoiding peanuts, had experienced their most recent reactions 5 ± 6 (mean $\pm$ SD) years (range, 0.5–10 years) before enrolment. All adult patients and the parents or guardians of minors signed informed consent. Minors who were > 6 years of age signed an assent. The University of Colorado Denver Institutional Review Board approved this study. Patients' reported symptoms after naturally occurring exposure to peanuts were classified into grades of anaphylaxis according to criteria established by the World Health Organization for evaluation of allergic reactions in the context of allergen-specific immunotherapy: grade I, cutaneous symptoms alone (angioedema, rash, hives); grade II, involvement of two organ systems (e.g. lower respiratory and cutaneous or gastrointestinal and lower respiratory); grade III, involvement of three or more organ systems and/or upper respiratory involvement (upper respiratory angioedema, stridor); grade IV, cardiovascular compromise (shock, decreased pulse, syncope) [41].

Proteins

Native Ara h 2 and Ara h 6 were purified as previously described [20].

Peptides for microarrays

A library of 77 peptides consisting of 20 amino acids (AAs) in length overlapping by 17 AAs (offset of 3) and covering the entire sequence of Ara h 2.01 as well as Ara h 6 were synthesized at the same time on solid support (80–90% purity). These peptides were then cleaved, diluted to 250–500 μ M in a DMSO-based buffer system, and immobilized on glass slides (JPT Peptide Technologies, Berlin, Germany) [42]. Random samples (5%) of the peptide library were analysed by liquid chromatography/mass spectrometry or matrix-assisted laser desorption/ionization. The PepStar™ peptide microarray platform (JPT Peptide Technologies), which places a proprietary 403 Dalton linker arm (Ttds-linker) at the C-terminus of each peptide as a spacer between the support and the reactive ligand, was used to generate customized peptide microarrays on glass slides as described by Shreffler [38]. Purified native Ara h 2 and native Ara h 6 were also immobilized within each array on the slides. Empty spots served as negative controls. Approximately 40% of the peptides (variable among the sera) had low IgE binding that was indistinguishable from the empty spots. Peptides found in other isoforms

of Ara h 2 and Ara h 6 were examined and had binding patterns that were similar to that seen with peptides derived from the parent isoform (data not shown).

Microarray printing

Triplicate spots were printed on APIX Protein Microarray Slides coated with a proprietary optically clear nitrocellulose (Intuitive Biosciences, Madison, WI, USA). All slides were stored at 4°C and used within 6 months from the date of manufacture.

Immunoassay

Slides were placed in a SIMplex™ 64-well device (Intuitive Biosciences) and blocked with PBS containing 0.05% Tween 20 (PBST) and 1% BSA. In preliminary experiments, we determined that the assay had a 10- to 1000-fold signal-to-noise ratio when the sera were diluted to give peanut-specific IgE values of 8–12 kU/L (data not shown). In the experiments reported here, sera were diluted in PBST with 0.1% BSA to give a final concentration of peanut-specific IgE of 9 kU/L (22 ng/mL), applied to each array, and incubated overnight at 4°C. Slides were washed, incubated with biotinylated murine monoclonal anti-human IgE (1 : 10 000) (Invitrogen, Carlsbad, CA, USA), washed again, and developed with anti-biotin gold conjugate followed by SilverQuant® Reagents A and B according to the manufacturer's instructions (Intuitive Biosciences). After incubation with buffer or the serum from our HIE patient (diluted 1 : 5), backgrounds were indistinguishable. Duplicate samples of each serum were assayed twice with three replicates within each array.

Analysis of microarray data

Peptides. The slides were scanned in an APIX Scanner (Intuitive Biosciences). Peptide-specific and background signals were determined. Z-scores were calculated as described by Lin and colleagues [43]. Briefly, the signal (S) for each spot containing a peptide was the log₂ transformation of the ratio of the median signal of the spot to the median signal of empty spots within the specific microarray. A z-score for each spot was determined using the equation [43]:

$$Z = \frac{S - \text{median (empty spots)}}{\text{median absolute deviation (empty spots)}}$$

The reported z-scores are the average of those from two independent assays carried out on separate days. Each assay included two arrays with triplicate spots of each peptide, duplicate spots of native Ara h 2 and Ara h 6, and 21 blank spots within each array. Binding to an individual peptide was considered to be positive if the z-score was > 3 [43]. A z-score of > 3 indicates that the

signal was significantly ($P < 0.003$) above the background [43]. For comparison, the data were also analysed by calculating the signal minus the mean of the background (empty spots) plus 3 or plus 4 standard deviations of the background data. In addition, the data were normalized based on relative binding to native Ara h 2 or Ara h 6 (for the appropriate peptides) within each array.

Epitopes. An IgE-binding epitope was defined as a region containing the same 6–13 amino acid sequence that was present in 3–5 contiguous peptides for which the mean z-score was > 3. This is similar to the approach described by Ayuso and colleagues [31].

Immunodominance. IgE-binding epitopes recognized by at least 50% of our peanut-allergic subjects were considered immunodominant. This is similar to previously published criteria [26, 44, 45].

Competition experiments. Microarray assay: A pool of four sera (D64, D70, D80, and D103; normalized by anti-peanut IgE content) was pre-incubated with a preparation containing native Ara h 2 and Ara h 6, then added to the microarray assay. These sera were chosen on the basis of having strong signals in the microarray assay. Subjects D64 and D80 reported clinical reactivity of grade II, whereas subjects D70 and D103 reported reactivity of grades IV and III, respectively (Table 1).

Cell release assay: Rat basophilic leukaemia (RBL-SX38) cells were grown, labelled with tritium-labelled serotonin (³H-5-HT), sensitized, and triggered with Ara h 2 or Ara h 6 as previously described [15]. For the competition experiments, cells were pre-incubated for 10 min with a 10 000–100 000 molar excess (limited by solubility) of purified 10–20-mer peptides containing dominant epitopes (described in the Results section) before addition of Ara h 2 or Ara h 6, and release of ³H-5-HT into the supernatant was measured as previously described [15].

Statistical analyses

In our initial analysis, we examined the raw signal for the binding of IgE to each peptide, the raw signal for the binding of IgE to each peptide normalized by binding to the parent molecule (Ara h 2 or Ara h 6), and binding of IgE to each peptide based on z-scores ranging from -2 to 28. Use of a validated statistical tool, a threshold of $z > 3$ for a positive signal, allowed us to analyse the data based on binary binding (yes, $z > 3$; no, $z \leq 3$) to individual peptides and binary binding (yes, $z > 3$; no, $z \leq 3$) to epitopes. We did this binary analysis because we hypothesized that the degree of binding of IgE to a peptide (size of the signal) is not as important as the fact that there is statistically significant binding.

Table 1. IgE values and grade of reaction

Subject	Total IgE (IU/ml)	Pnt IgE (kU/L)	Rxn Grade
D19	136	59	2
D44	1015	65.3	3
D48	138	71.1	2
D50	538	37.8	3
D53	1185	74.7	3
D59	290	13.4	3
D60	454	159	4
D61	161	64	1
D63	3263	78.6	3
D64	2803	591	2
D65	2421	180	3
D66	186	77	3
D67	568	137	3
D68	212	25.2	4
D69	212	22.3	4
D70*	1423	500	2
D70B*	245	52.7	4
D71	4939	88.4	2
D72	474	14.1	1
D74	194	28.9	3
D77	505	48.7	3
D78	1325	62.6	2
D80	545	164	2
D81	268	67.5	3
D82	265	18.9	3
D98	80.5	16.1	4
D103	1797	787	3
D105	88.2	14	2
D107	199	65.3	3
D114	117	22.4	1

*D70 and D70B are not related.

After generating the final microarray data, heat maps and unsupervised hierarchical cluster analyses were performed with R-2.14.1 software for Windows (<http://cran.r-project.org/bin/windows/base/>) to determine whether patients sorted into clusters based on clinical history. GraphPad Prism 5.0c for the Macintosh (GraphPad, La Jolla, CA, USA) was used to generate graphs and for statistical analysis. The following tests were used: Spearman rank-order correlation coefficients for correlations; Mann-Whitney test for comparisons of the number of binding events; and Fisher's exact test for comparing frequencies of two possible outcomes. All comparisons were two-tailed. Where multiple comparisons were performed, the Bonferroni correction was used and corrected *P*-values denoted as *p*_c. *P*-values of < 0.05 were considered to be significant.

Results

Clinical data

Sera from 30 peanut-allergic individuals were assessed. Serologic data and grades based on reported symptoms

for each subject are shown in Table 1 with the subjects listed numerically to allow cross-referencing to other studies using some of the same subjects. Reported symptoms and grades of anaphylaxis for each subject are shown in Table S1. Three subjects (10%) were classified as grade I, 8 (27%) as grade II, 14 (47%) as grade III, and 5 (17%) as grade IV. The total IgE, peanut-specific IgE, the ratio of peanut IgE to total IgE, and the intensity of binding of IgE against Ara h 2 and Ara h 6 had no discernible relationship to the severity of the reported clinical reactions to peanuts (data not shown).

Identification of IgE-binding fingerprints to peptides of Ara h 2 and Ara h 6 for each individual

For each of the 30 sera, we determined the intensity of binding to the individual peptides, intact native Ara h 2 and Ara h 6, and blank spots (all on the array). Then, we calculated *z*-scores. Each subject had a distinctive IgE-binding configuration: a fingerprint for the peptides of Ara h 2 and Ara h 6. *Z*-score data from 4 subjects, one with each grade of anaphylaxis, are shown in Fig. 1. The threshold for positive binding, a *z*-score > 3, is shown as a horizontal line on each graph. Colour-coding in this and subsequent figures and tables denotes peptides containing specific epitopes.

Data for these 4 subjects are again presented in Fig. S1A (Ara h 2) and Fig. S1B (Ara h 6), in which the data are expressed as *z*-scores (Fig. S1A and S1B; graphs A, E, I, and M), as mean values minus the mean of the background +3 SD of the background (Fig. S1A and S1B; graphs B, F, J, and N), or as mean values minus the mean of the background +4 SD of the background (Fig. S1A and S1B; graphs C, G, K, and O). Data are also presented after further normalization for binding to native Ara h 2 or native Ara h 6 for the Ara h 2 and Ara h 6 peptides, respectively (Fig. S1A and S1B; graphs D, H, L, and P). Regardless of the method of expressing the data, the patterns are consistent.

Binding values for each of the 30 sera for each of 41 peptides of Ara h 2 and for native Ara h 2 are shown in Tables S2A–F; binding values for 36 peptides of Ara h 6 and for native Ara h 6 are shown in Tables S3A–F. Even after adjusting the sera to contain the same amounts of peanut-specific IgE before each assay (see Materials and methods), there is considerable variability in the maximal binding to peptides with different sera. This variability was not reduced by further adjusting the binding based on that to native Ara h 2 or native Ara h 6 (data not shown).

Hierarchical analyses of IgE binding to peptides

To analyse the binding of IgE to these peptides, we generated group-peptide plots for binding to Ara h 2

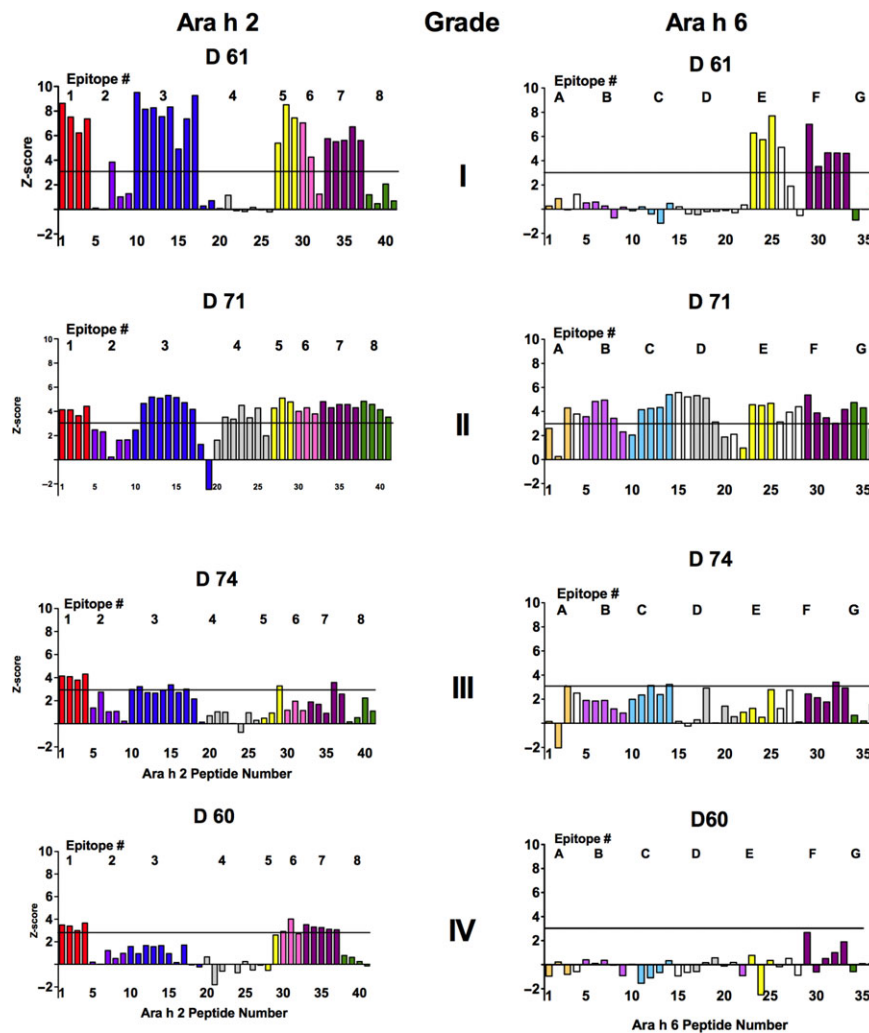


Fig. 1. Microarray analysis of IgE binding to overlapping 20-mer peptides of Ara h 2, left, and Ara h 6, right. Sera are from 4 of 30 representative patients with varying histories regarding the severity of reactivity. From top to bottom: D61, grade I (less severe); D71, grade II; D74 grade III; and D60, grade IV (most severe). Linear peptide regions that bind peanut-specific IgE are colour-coded (see Fig. 2). The Y axis is a calculated z-score (see Methods). The established cut-off score of $z = 3$ is shown as a horizontal line.

(Fig. S2A) and Ara h 6 (Fig. S2B) and performed an unsupervised hierarchical cluster analysis for the 30 subjects using the intensity of IgE binding (z-scores) to each peptide to Ara h 2 (Fig. S3A) and Ara h 6 (Fig. S3B). Patients with relatively severe clinical histories (shown in red numbers) did not significantly cluster into specific groups although, at the bottom of the heat map for Ara h 6, there appeared to be clustering of data from subjects with more severe clinical histories (Fig. S3B).

Analysis based on a binary (yes or no) binding to peptides

We therefore re-examined our data based on positive or negative binding (binary; yes, $z > 3$ or no, $z \leq 3$) to the peptides of Ara h 2 (Fig. S4A) and Ara h 6 (Fig. S4B). We also performed unsupervised cluster analyses for Ara h 2

(Fig. S5A) and Ara h 6 (Fig. S5B). Using this dichotomous approach for IgE binding to peptides, there was a suggestion that subjects clustered into patterns, but we did not find any statistically significant relationships.

Ara h 2 and Ara h 6 share homologous linear epitopes

As stated in Materials and Methods, an IgE-binding epitope was defined as a region containing the same 6–13 amino acid sequence that was present in 3–5 contiguous peptides for which the mean z-score was > 3 . For example, in Fig. 1, the binding pattern for serum D61 for Ara h 2 shows that there is binding to epitopes 1, 3, 5, 6, and 7 (see Fig. 2). These were determined to be epitopes because the average z-score for binding to peptides that contain a specific epitope (noted by different colours) was > 3 . Overall, when all 30 sera were examined, eight

IgE-binding epitopes were identified for Ara h 2 (Fig. 2a), which is consistent with published data [38, 46]. These epitopes of Ara h 2 are notated here as epitopes 1–8 (Fig. 2b). Seven novel IgE-binding epitopes were identified for Ara h 6 (Fig. 2a); these are notated as epitopes A–G (Fig. 2b). Three-dimensional images of these epitopes in the context of the intact molecule are shown in Fig. 2c and Fig. 2d for Ara h 2 and Ara h 6, respectively.

As shown in Fig. 2a, two epitopes of Ara h 6 (A and C) are unique in that they have no significant homology to Ara h 2, whereas 5 epitopes (B, D, E, F, and G) are highly homologous (70–93%) to epitopes of Ara h 2 (2, 4, 5, 7, and 8, respectively). Epitope 6 in Ara h 2 shares homology to a corresponding sequence in Ara h 6 (67%). However, given a lack of significant IgE binding (mean z-score < 3), this region in Ara h 6 was not identified as an IgE-binding epitope.

Spearman rank correlation coefficients of IgE binding revealed a modest correlation for binding to epitope 1 of Ara h 2 and epitope A of Ara h 6 (Fig. 3a; homology = 0%; $r = 0.37$, $P = 0.04$, $p_c = \text{ns}$); a strong

correlation for binding to epitope 2 of Ara h 2 and epitope B of Ara h 6 (Fig. 3b; homology = 81%; $r = 0.55$, $p_c = 0.01$); no significant correlation for binding to epitopes 3 of Ara h 2 and epitope C of Ara h 6 (Fig. 3c; homology = 0%; $r = 0.21$, $p_c = \text{ns}$); and strong correlations for binding to epitope 4 of Ara h 2 and epitope D of Ara h 6 (Fig. 3d; homology = 70%; $r = 0.64$, $p_c < 0.01$), epitope 5 of Ara h 2 and epitope E of Ara h 6 (Fig. 3e; homology = 92%; $r = 0.65$, $p_c < 0.01$), epitope 7 of Ara h 2 and epitope F of Ara h 6 (Fig. 3f; homology = 83%; $r = 0.82$, $p_c < 0.001$), and epitope 8 of Ara h 2 and epitope G of Ara h 6 (Fig. 3g; homology = 85%; $r = 0.90$, $p_c < 0.001$).

IgE binds to more linear epitopes of Ara h 2 than to those of Ara h 6

Linear epitopes are not able to cross-link IgE–FcεRI complexes [47, 48] and yet may be part of larger conformational complexes. We reasoned that the presence or absence of binding (yes or no) beyond a set

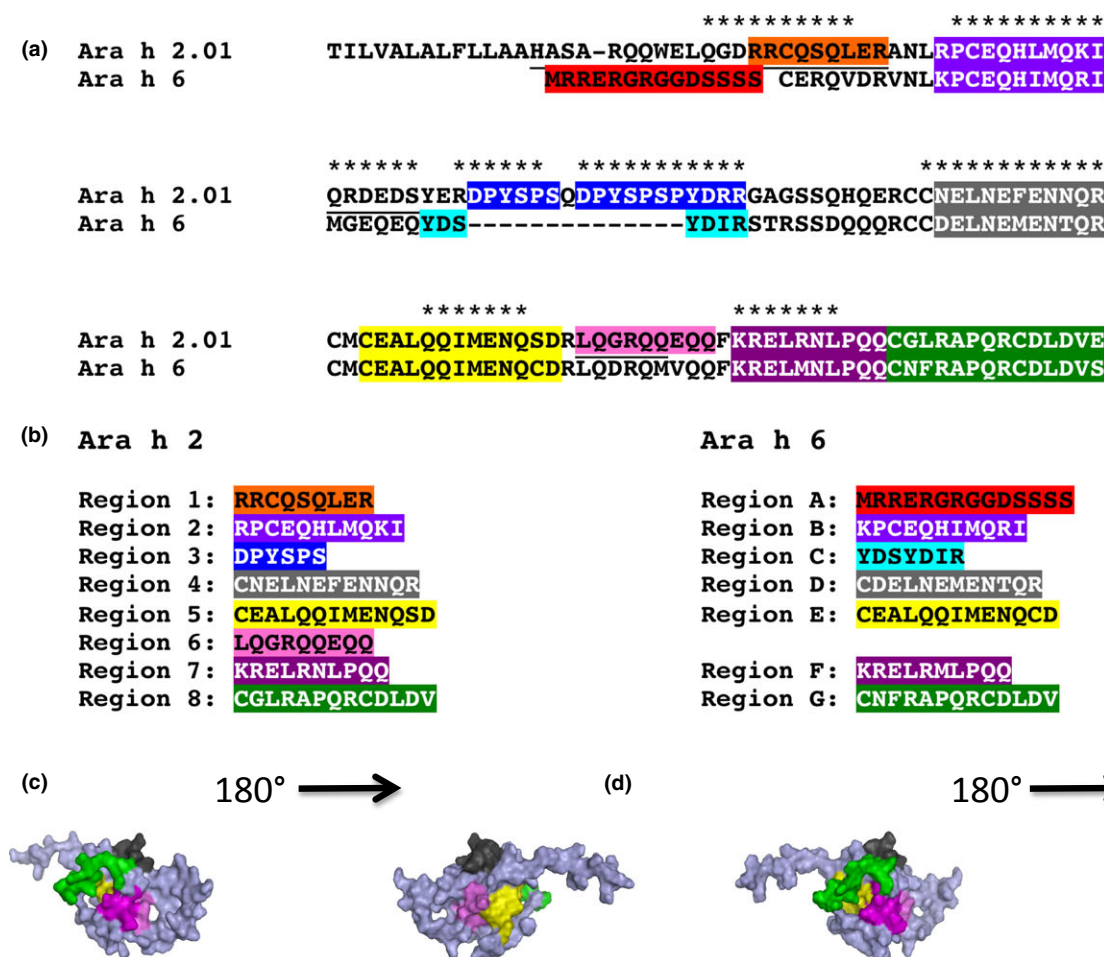


Fig. 2. Amino acid alignment is shown for Ara h 2 and Ara h 6 (a). Different colours denote epitopes for Ara h 2 and Ara h 6; these are listed (b). The asterisks (*) in A, denote linear sequences for Ara h 2 that have been previously identified [38, 46]. A colour-coded 3-D image is shown (c).

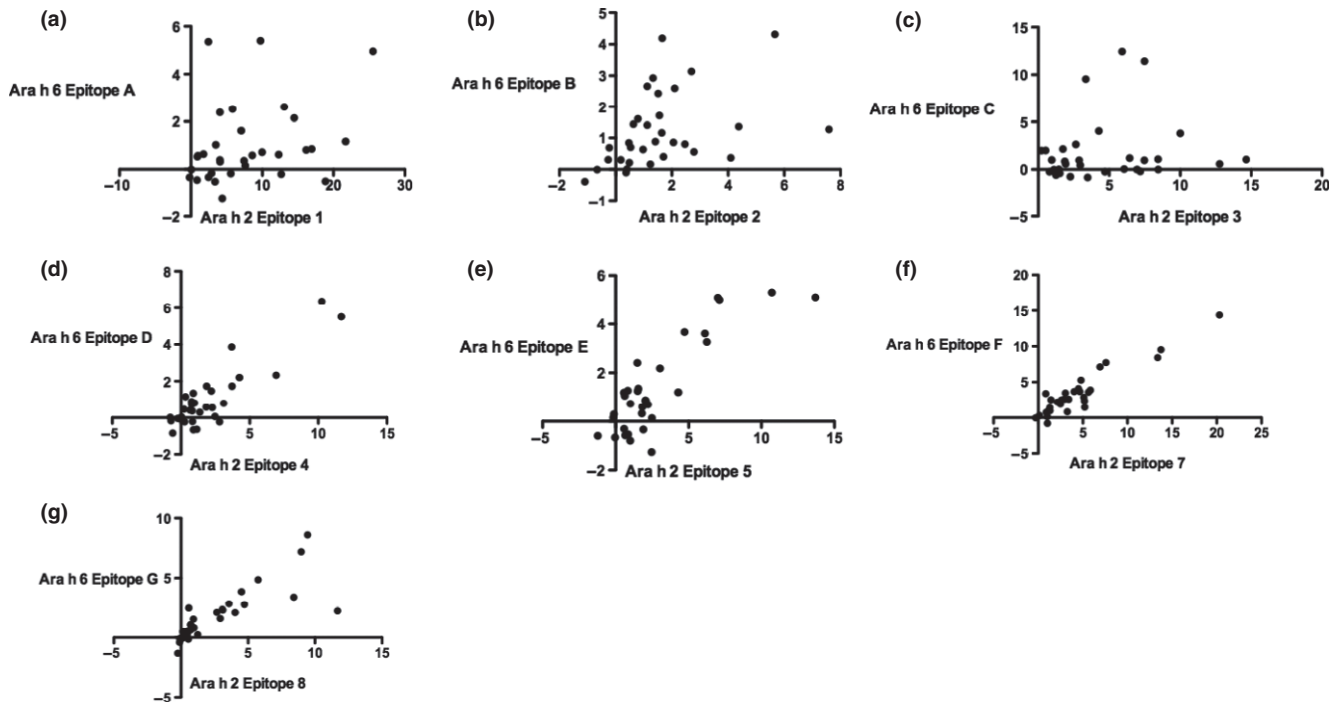


Fig. 3. Correlation of IgE binding to linear epitopes. Spearman correlation analysis was performed with z-scores for each homologous pair of epitopes of Ara h 2 and Ara h 6 (horizontal and vertical axes, respectively): epitope 1 of Ara h 2 and epitope A of Ara h 6 (a, $r = 0.37$, $P = 0.04$, $p_c = \text{ns}$); epitope 2 of Ara h 2 and epitope B of Ara h 6 (b, $r = 0.55$, $p_c = 0.01$); epitope 3 of Ara h 2 and epitope C of Ara h 6 (c, $r = 0.21$, $p_c = \text{ns}$); epitope 4 of Ara h 2 and epitope D of Ara h 6 (d, $r = 0.64$, $p_c < 0.01$); epitope 5 of Ara h 2 and epitope E of Ara h 6 (e, $r = 0.65$, $p_c < 0.01$); epitope 7 of Ara h 2 and epitope F of Ara h 6 (f, $r = 0.82$, $p_c < 0.001$); and epitope 8 of Ara h 2 and epitope G of Ara h 6 (g, $r = 0.90$, $p_c < 0.001$).

threshold (z-score > 3) would be more informative than the relative intensities of binding to specific epitopes. Accordingly, we next examined our data based on positive or negative binding to linear epitopes rather than binding to peptides. We then generated group-epitope heat maps for Ara h 2 (Fig. 4a) and for Ara h 6 (Fig. 4b). Viewing the binding of IgE to linear epitopes in this way, we found that, regardless of clinical history, subjects' IgE had more frequent positive binding ($z > 3$) to linear epitopes of Ara h 2 (Table 2A) [median = 3 epitopes/serum (E/S)] than to the linear epitopes of Ara h 6 (Table 2B) (median = 1 E/S; $P < 0.001$). In Tables 2A and B, a 'yes' or 'Y' corresponds to a yellow box; the absence of 'Y' denotes lack of significant binding and corresponds to a blue box in Figs 4 and 5.

Because 30 sera could potentially bind to each of 8 epitopes of Ara h 2 and 7 epitopes of Ara h 6, we determined that there were, respectively, 240 and 210 potential binding events (BEs) for Ara h 2 and Ara h 6. For all 30 sera, there were 99 BEs for Ara h 2 (Table 2A) compared to 38 for Ara h 6 (Table 2B) ($P < 0.0001$). For Ara h 2, epitopes 1 ($z > 3$ for 23 sera), 3 ($z > 3$ for 18 sera), and 7 ($z > 3$ for 17 sera) are immunodominant. For Ara h 6, only epitope F ($z > 3$ for 15 sera) was immunodominant.

The frequency of IgE binding to linear epitopes of Ara h 2 and Ara h 6 corresponds to clinical histories

Characteristic patterns emerged when subjects' IgE binding to linear epitopes was examined in the context of their histories of reaction to peanut exposure. For Ara h 2 (Table 2A), we found 52 BEs (34%) of 152 (19X8 epitopes) possible BEs for the 19 subjects with more severe histories (grades III + IV) compared to 47 BEs (53%) of 88 (11X8 epitopes) possible BEs (53%) for the 11 subjects with less severe histories (grades I + II) ($P < 0.01$). For Ara h 6 (Table 2B), we found only 16 BEs (12%) of 133 (19X7 epitopes) possible BEs for the 19 subjects with grades III + IV compared to 25 BEs (32%) of 77 (11X7 epitopes) possible BEs for the 11 subjects with histories of grade I + II reactivity ($P < 0.001$). When specific epitopes are examined individually, IgE from subjects with less severe histories (grades I and II) bind epitope 5 of Ara h 2 more frequently (7 of 11 sera) than does IgE from subjects with more severe histories (2 of 19 sera) ($P = 0.004$, $p_c = 0.03$) (Table 2A). A similar trend was seen with binding to epitope G of Ara h 6 (Table 2B), but this did not achieve statistical significance after correction for multiple comparisons. IgE from 4 of 11 patients with

Table 2. A Binary binding of IgE to epitopes of (A) Ara h 2 (B) Ara h 6

		Epitope							
Symptom	Serum	1	2	3	4	5	6	7	8
(A)									
Grade I	D61	Y		Y		Y	Y	Y	
	D72								
Grade II	D114	Y	Y		Y	Y			Y
	D48	Y		Y	Y	Y	Y	Y	Y
	D64		Y	Y		Y	Y	Y	Y
	D70	Y		Y				Y	
	D71	Y		Y	Y	Y	Y	Y	Y
	D78	Y		Y			Y		
	D80			Y			Y	Y	
	D105	Y			Y	Y			
Grade III	D19	Y			Y	Y		Y	Y
	D44	Y		Y	Y		Y	Y	Y
	D50							Y	Y
	D53								
	D59								
	D63			Y					
	D65	Y					Y		
	D66	Y		Y					
	D67	Y		Y				Y	Y
	D74	Y							
Grade IV	D77	Y		Y					
	D81	Y	Y	Y				Y	
	D103	Y		Y			Y		
	D107	Y		Y		Y	Y	Y	
	D82	Y	Y	Y				Y	
	D60	Y					Y	Y	
	D68	Y							
	D69	Y							
	D70B	Y		Y	Y	Y	Y	Y	Y
	D98	Y		Y				Y	Y
		Epitope							
Symptom	Serum	A	B	C	D	E	F	G	
(B)									
Grade I	D61					Y	Y		
	D72								
Grade II	D114					Y			
	D48		Y	Y		Y	Y	Y	
	D64		Y			Y	Y	Y	
	D70						Y		
	D71		Y	Y	Y	Y	Y	Y	
	D78	Y							
	D80	Y		Y			Y		
	D105								
Grade III	D19						Y	Y	
	D44	Y			Y		Y	Y	
	D50						Y		
	D53								
	D59								
	D63								
D65									

(continued)

Table 2 (continued)

Symptom	Serum	Epitope							
		A	B	C	D	E	F	G	
	D66			Y					
	D67								
	D74								
	D77								
	D81								
	D103								
	D107						Y		
	D82								
	D60								
	D68							Y	
Grade IV	D69								
	D70B				Y	Y	Y		
	D98			Y				Y	

less severe histories (grades I and II) bind epitope G of Ara h 6 compared to 1 of 19 subjects with more severe histories (grades III and IV) but this was not statistically significant ($P = 0.047$, $p_c = 0.33$). Thus, IgE from patients with relatively more severe clinical histories recognize fewer linear epitopes of both Ara h 2 and Ara h 6 when the epitopes are viewed as a whole.

Hierarchical analyses of IgE binding to linear epitopes

We also performed an unsupervised hierarchical cluster analysis (Fig. 5). For Ara h 2 (Fig. 5a), 6 of 8 subjects who clustered together into nodes 1 and 2A had a history of grade I or II severity whereas, of the 22 remaining subjects who did not cluster to these nodes, 5 had grades I or II severity (Figs 5a and 6a; $P = 0.028$). For Ara h 6 (Fig. 5b), the largest single node, 2B2B1, contained 17 subjects, 14 of which had a history of grade III or IV severity. Of the 13 subjects who did not cluster into this node, 5 had severity grades of III or IV (Figs 5b and 6b; $P = 0.02$). Thus, subjects with milder symptoms (grades I and II) clustered into nodes 1 and 2A of Ara h 2 node, while those with more severe symptoms (grades III and IV) clustered into node 2B2B1 of Ara h 6. Moreover, of the 17 individuals who clustered to node 2B2B1 of Ara h 6, only one subject, D105, clustered to either node 1 or 2A of Ara h 2, as compared with 16 who did not (Figs 5a, b, and 6c; $P < 0.01$).

Node 2B2B1 of Ara h 6 is characterized by lack of significant ($z \leq 3$) binding to all epitopes of Ara h 6 or to only epitope F and is populated predominantly by individuals having a history of grades III or IV severity (Figs 5b and 6b). Taken together, subjects who cluster into this node do not cluster into nodes 1 and 2A of Ara h 2, which are characterized by significant ($z > 3$) binding to epitope 4 or to epitopes 2 and 5 of Ara h 2 and are predominantly populated by those having a

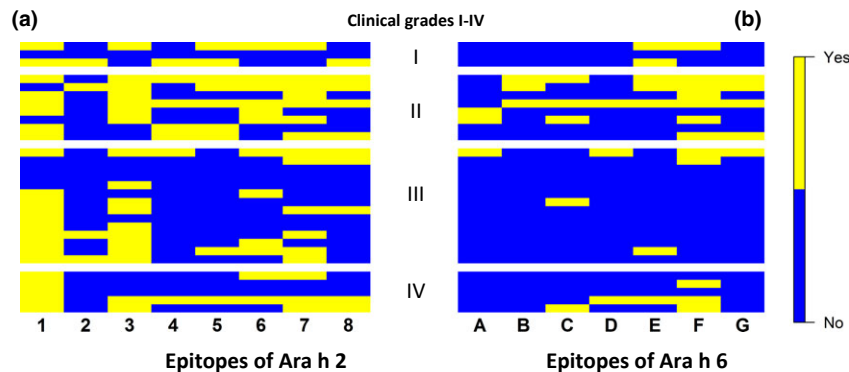


Fig. 4. Heat maps of binary (yes or no; $z \leq 3/z > 3$) binding of IgE to epitopes for Ara h 2 (a) and Ara h 6 (b) for all 30 sera sorted according to grades of reaction based on clinical histories. 'yes' is denoted by yellow and 'no' is denoted by blue.

history of grade I or II severity (Figs 5a, 6a). Thus, we have tentatively identified a high-risk pattern in which subjects with allergies having high grades of severity cluster to node IB3B of Ara h 6 but not to nodes 1 or 2A of Ara h 2. As shown in Fig. 6d, 16 subjects had this high-risk pattern; of these, 14 had a history of grades III or IV severity, whereas only two had a history of grade I or II severity. Moreover, of the 14 subjects who did not demonstrate this high-risk pattern, nine had grade I or II severity (OR = 12.6; 95% CI = 2.0–79.5; $P < 0.01$).

Competition experiments with linear epitopes

To determine whether the binding of IgE to peptides in the microarray was specific, a pool of 4 sera, D64, D70, D80, and D103, normalized by anti-peanut IgE content, was pre-incubated with native Ara h 2 or native Ara h 6. This resulted in > 90% inhibition of binding to their respective peptides (data not shown).

We also considered that the identified linear epitopes of Ara h 2 and Ara h 6 might effectively block IgE cross-linking of FcεRI and resultant activation of mast cells. In that, Albrecht and colleagues [48] have shown that peptides of Ara h 2 were unable to block cross-linking of IgE and cell activation by Ara h 2, and we performed similar experiments only with Ara h 6. In separate assays, we sensitized RBL SX-38 cells with IgE from each of three patients (D19, D64, and D70) with a history of mild reactions (grade I or II) and evidence of predominant binding to epitopes E, F, and G of Ara h 6. We pre-incubated these sensitized cells with 10 000 molar excess of epitope E (limited by solubility) plus a 100 000 molar excess of epitopes F and G and then added Ara h 6 as described in Materials and Methods. We were unable to detect a significant inhibitory effect (data not shown).

Discussion

In this study, we used peptide microarrays to examine IgE binding to linear peptides and then to epitopes of

the highly potent peanut allergens, Ara h 2 and Ara h 6, for 30 subjects with strong histories and serologic evidence of clinically important peanut allergy. The eight epitopes identified for Ara h 2, that are similar to those previously reported, include the epitopes that have been recently shown to be responsible for cross-reactivity with Ara h 1 and Ara h 3 [38, 46, 49]. Seven epitopes were newly identified for Ara h 6 (Fig. 2); six of these showed positive correlations for IgE binding to homologous epitopes of Ara h 2 ($P < 0.001$) (Fig. 3). Unexpectedly, although it was not true for all sera, the intensity of binding to peptides of Ara h 2 was generally greater than the intensity of binding to peptides of Ara h 6 (Figs 3, S1, S2, S3, S4, and S5).

Given that binding of IgE to denatured proteins (e.g. on immunoblots) does not reflect the importance of IgE to cell activation following allergen-IgE cross-linking (discussed in Zhuang and Dreskin, [21]), we hypothesized that the absolute or relative extent of binding of IgE to peptides may not accurately reflect the importance of that peptide's contribution to the clinically important phenomenon of allergen-IgE cross-linking. For this reason, we focused on a dichotomous model in which a specific serum either did or did not have positive binding to a specific linear epitope. We defined a positive signal for an epitope if > 50% of the peptides containing the putative epitope gave z -score of > 3 (see Materials and Methods).

Overall, individual IgE samples bound to linear epitopes of Ara h 2 more frequently than to epitopes of Ara h 6 ($P < 0.002$) (Fig. 4; Table 2). This was unexpected in that there is significant homology between IgE-binding linear epitopes of these two molecules, as well as significant correlation of IgE binding (Fig. 2 and Fig. 3). We interpret this as evidence that conformational epitopes may be more important for Ara h 6 than for Ara h 2. We hypothesize that Ara h 6 is more tightly folded than Ara h 2 and that this leads to conformational IgE-binding epitopes that are less cross-reactive with the linear epitopes that may contribute to the conformational epi-

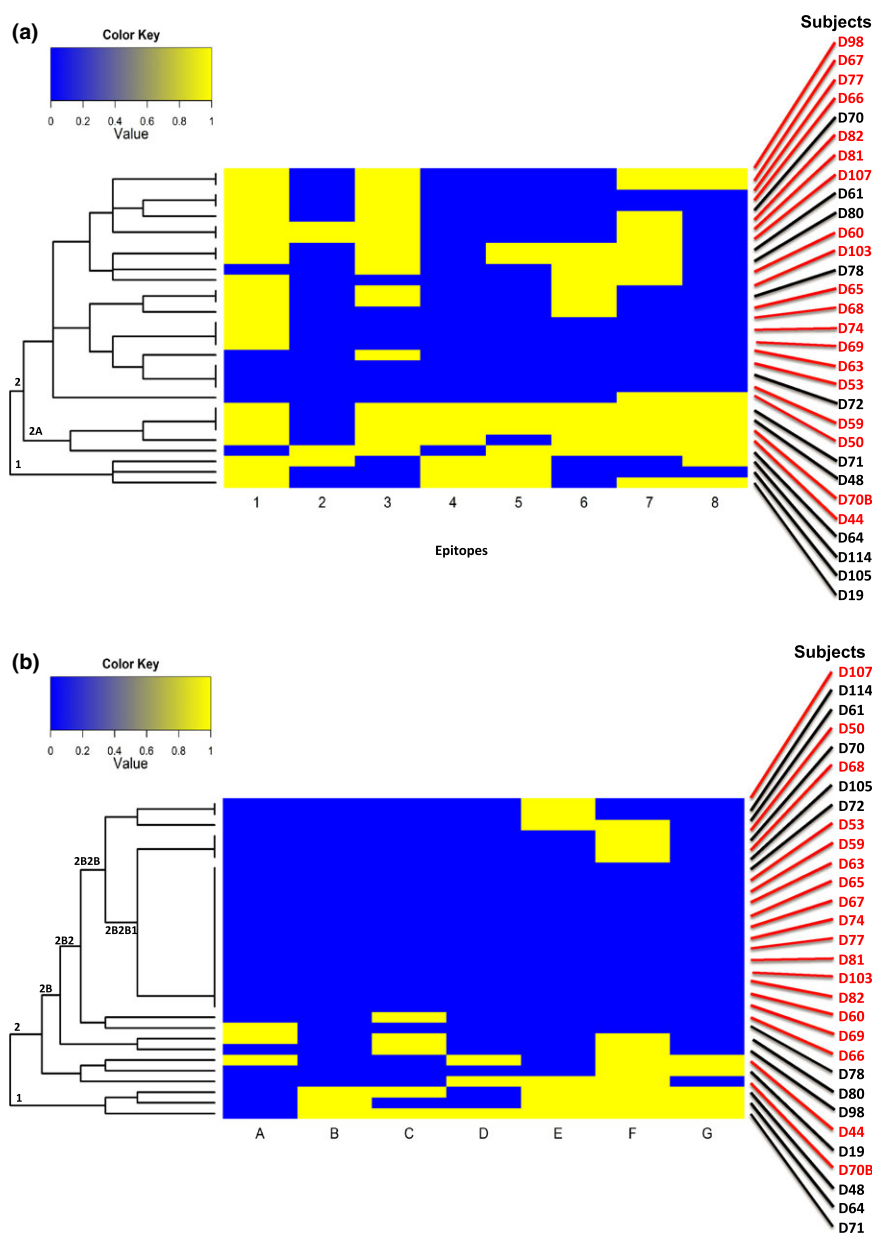


Fig. 5. Unsupervised cluster analysis of the data from Fig. 4 for Ara h 2 (a) and Ara h 6 (b). 'yes' is denoted by yellow; 'no' is denoted in blue. Sera from subjects with a history of grade I or II reactivity are in black. Those with a history of grade III or IV reactivity are in red.

topes. This also explains the observation that the intensity of IgE binding to the peptides that contain the linear epitopes is also less for Ara h 6 than for Ara h 2. This is compatible with the observation that Ara h 6 has 5 disulphide bonds compared with 4 disulphide bonds for Ara h 2 and with our experience that proper folding of recombinant Ara h 6 is particularly important to preserve allergenic activity [21].

Also unexpectedly, IgE from subjects with histories of relatively severe allergic responses to peanut exposure recognized a significantly more limited breadth of linear epitopes of Ara h 2 and Ara h 6 than did those

from subjects who reported less severe responses to peanuts (Table 2). This result is contrary to several reports of more binding of IgE to linear epitopes of allergens with IgE from patients with severe histories [26, 29, 32, 38]. However, it is compatible with reports of a positive relationship between the amount of IgE binding to linear epitopes and allergen-specific IgE concentrations in the sera and no relationship to clinical history [31, 39, 40]. The most likely explanations of this finding are that, in contrast to most previous reports of IgE binding to peptides in microarrays [26, 29, 31, 32, 38, 39, 50], we adjusted our sera to the

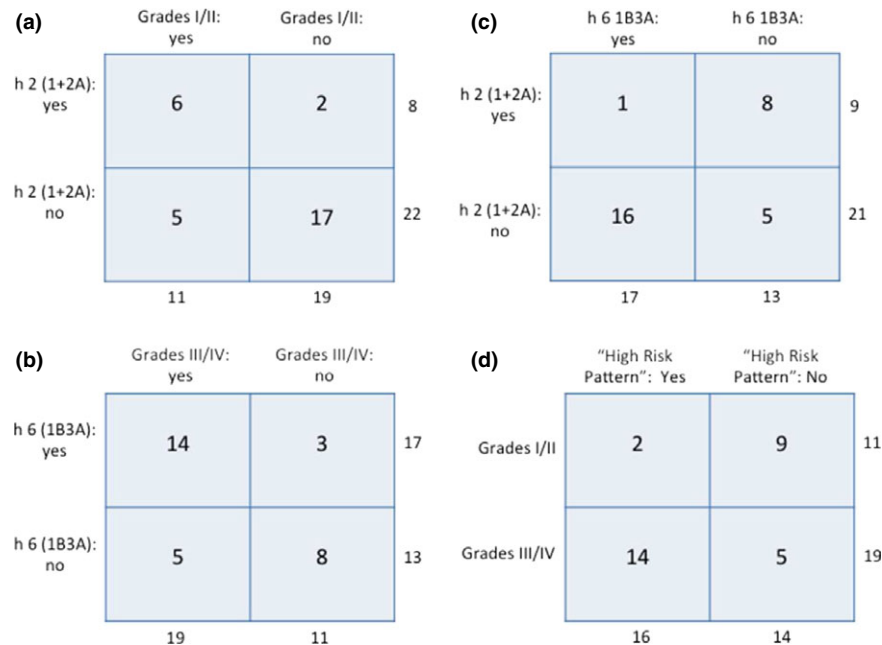


Fig. 6. Contingency plots of key data from the unsupervised cluster analysis shown in Fig. 5. Totals for each horizontal and vertical set of numbers are shown. For all plots, $n = 30$. (a) Frequency of subjects with a history of grades I or II severity cluster in either node 1 or 2A of Ara h 2 ($P = 0.03$); (b) frequency of subjects with a history of grades III or IV severity cluster in node 2B2B1 of Ara h 6 ($P = 0.02$); (c) frequency of subjects who cluster in node 2B2B1 of Ara h 6 and do not cluster in either node 1 or 2A of Ara h 2 ($P < 0.01$); and (d) frequency of subjects having the high-risk pattern [h 6 (2B2B1), yes and h 2 (1 + 2A), no] ($P < 0.01$).

same content of anti-peanut IgE to avoid bias due to differences in peanut-specific IgE levels and used a dichotomous model (discussed above) to determine statistically significant binding. Normalization based on peanut-specific IgE levels removes the confounding variable of differences in peanut-specific IgE among the samples. For example, without this normalization, sera with very high IgE levels may have statistically significant, but biologically unimportant, binding to minor epitopes. Of note, we found that further normalization of our data based on binding of IgE to native Ara h 2 and/or Ara h 6 within each microarray assay did not substantially change the final data (Fig. S1).

These observations that point towards the importance of conformational as opposed to linear epitopes are in line with recent observations that conformational epitopes rather than linear epitopes are critical for binding of IgE and for activation of IgE-sensitized basophils and/or mast cells [48, 51, 52]. As reported by Albrecht and colleagues [48], peptides of Ara h 2 were unable to block cross-linking of IgE and cell activation by Ara h 2. In our hands, peptides containing epitopes of Ara h 6 were unable to block IgE-mediated activation of sensitized RBL SX-38 cells by Ara h 6 (data not shown). Because Ara h 2 and Ara h 6 are stable to digestion, it is likely that conformational epitopes are important with regard not only to immediate reactivity (as is the experimental

set-up for these studies), but also following ingestion and exposure to the digestive track. We propose that conformational epitopes are important for all IgE cross-linking activity by Ara h 2 and Ara h 6 and are most important for patients with relatively severe reactions. These epitopes likely have substantially greater affinity for IgE binding than do linear epitopes.

Finally, in an unsupervised hierarchical cluster analysis, we have tentatively identified a novel high-risk pattern in which patients with high grades of severity cluster to node 2B2B1 of Ara h 6 (Fig. 5b) but not to nodes 1 or 2A of Ara h 2 (Figs 5a and 6d). Of note, node 2B2B1 of Ara h 6 is characterized by either binding only to epitope F (the only epitope of Ara h 6 that met our criteria of immunodominance) or no detectable binding above our predetermined threshold ($z > 3$) to any IgE-binding linear epitopes of Ara h 6 (Fig. 5b).

Weaknesses of this study include the use of historical clinical data rather than data obtained by double-blind placebo-controlled food challenge and the modest number of patients. Based on the data presented here, a *post hoc* power analysis (not shown) reveals that the analysis of the 'high-risk pattern' required only 24 subjects to have 90% power. Another issue is that the 'high-risk pattern' is associated with lower binding to Ara h 6. This can only be assessed in the context of control sera that have high levels of binding to both Ara h 2 and

Ara h 6. Even given these limitations, our approach has yielded dramatic findings that may have significant clinical utility. However, this final observation needs to be repeated with an independent cohort.

In conclusion, subjects with a history of relatively severe clinical reactions have characteristic patterns of binding to linear epitopes of Ara h 2 and Ara h 6, which may be useful in predicting clinical reactions to future exposure to peanuts. The overall approach of focusing on IgE-binding epitopes of the most potent allergens identified in functional assays may also be useful for other IgE-mediated food allergies.

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Author contributions

Dr. Dreskin and Dr. Otsu conceived and designed the experiments. Dr. Otsu did the microarray assays. Grants to Dr. Dreskin and funds raised by Dr. Dreskin funded the project. Dr. Guo performed the heat map analysis and assisted in statistical analysis. All three authors contributed to analysis of the data and to writing the manuscript.

Conflict of interest

Drs. Dreskin and Otsu have a patent pending based on methodology described in this manuscript. Dr. Guo does not have any conflict of interests to disclose regarding this manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Demographics, clinical history, and grade of reaction.

Table S2A. Ara h 2. Intensities for sera #1–5.

Table S2B. Ara h 2. Intensities for sera #6–10.

Table S2C. Ara h 2. Intensities for sera #11–15.

Table S2D. Ara h 2. Intensities for sera #16–20.

Table S2E. Ara h 2. Intensities for sera #21–25.

Table S2F. Ara h 2. Intensities for sera #26–30.

Table S3A. Ara h 6. Intensities for sera #1–5.

Table S3B. Ara h 6: Intensities for sera #6–10.

Table S3C. Ara h 6: Intensities for sera #11–15.

Table S3D. Ara h 6: Intensities for sera #16–20.

Table S3E. Ara h 6: Intensities for sera #21–25.

Table S3F. Ara h 6: Intensities for sera #26–30.

Figure S1A. Further analysis of IgE binding to overlapping 20-mer peptides of Ara h 2.

Figure S1B. Further analysis of IgE binding to overlapping 20-mer peptides of Ara h 6.

Figure S2. Heat maps of IgE-binding to individual peptides (z-scores) for peptides of Ara h 2 (a) and Ara h 6 (b).

Figure S3A. Unsupervised cluster analysis of the data from Figure S2 for Ara h 2.

Figure S3B. Unsupervised cluster analysis of the data from Figure S2 for Ara h 6.

Figure S4. Heat maps of binary (yes or no; $z \leq 3/z > 3$) binding of IgE to peptides of Ara h 2 (a) and Ara h 6 (b).

Figure S5A. Unsupervised cluster analysis of the data from Figure S4 for Ara h 2.

Figure S5B. Unsupervised cluster analysis of the data from Figure S4 for Ara h 6.

Table S1. Demographics, clinical history, and grade of reaction

Subject	Gen-der	Age	Race/ Ethnicity*	Age at onset	Total IgE (IU/ml)	Pnt IgE (kU/L)	Hives	Rash	AE [#]	UR [#]	LR [#]	GI [#]	↓BP [#]	Rxn Grade
D19	M	29	W	11M	136	59	Y	N	N	N	Y	N	N	2
D44	M	14	W	1Y 6M	1015	65.3	N	N	Y	Y	Y	Y	N	3
D48	M	18	W	UNK	138	71.1	Y	Y	Y	N	Y	N	N	2
D50	M	11	H	1Y 1M	538	37.8	Y	Y	Y	N	Y	Y	N	3
D53	M	11	W	2Y	1185	74.7	Y	N	Y	N	Y	Y	N	3
D59	M	10	W	3Y	290	13.4	Y	N	Y	N	Y	Y	N	3
D60	M	16	W	3Y	454	159	Y	Y	Y	N	Y	N	Y	4
D61	F	7	W	2Y	161	64	Y	Y	Y	N	N	N	N	1
D63	F	19	A	5Y	3263	78.6	Y	Y	Y	Y	Y	Y	N	3
D64	F	9	W	7M	2803	591	Y	Y	Y	N	Y	N	N	2
D65	F	11	W	1Y 6M	2421	180	N	N	Y	Y	Y	N	N	3
D66	M	7	W	1Y	186	77	N	N	N	N	Y	Y	N	3
D67	M	9	W	2Y 4M	568	137	Y	N	Y	N	Y	Y	N	3
D68	F	16	W	3Y	212	25.2	Y	Y	Y	N	Y	Y	Y	4
D69	M	12	W	1Y 1M	212	22.3	Y	Y	Y	N	Y	N	Y	4
D70 [‡]	M	13	W	1Y 4M	1423	500	Y	N	Y	N	Y	N	N	2
D70B [‡]	M	7	W	1Y	245	52.7	Y	Y	Y	N	Y	Y	Y	4
D71	M	7	A/AA	2Y 6M	4939	88.4	Y	N	N	N	N	Y	N	2
D72	M	10	W	1Y 4M	474	14.1	Y	Y	Y	N	N	N	N	1
D74	F	15	H	2Y	194	28.9	Y	Y	Y	N	Y	N	N	3
D77	M	11	W	4M	505	48.7	Y	Y	N	Y	Y	Y	N	3
D78	M	18	W	16Y	1325	62.6	N	Y	Y	N	N	N	N	2
D80	M	13	W	2Y 6M	545	164	N	N	N	N	N	Y	N	2
D81	M	16	W	2Y	268	67.5	Y	Y	Y	N	Y	Y	N	3
D82	F	18	W	3Y 6M	265	18.9	Y	Y	Y	Y	N	N	N	3
D98	F	26	W	4Y	80.5	16.1	Y	Y	Y	Y	Y	Y	Y	4
D103	F	9	W	1Y 6M	1797	787	Y	Y	Y	N	Y	Y	N	3
D105	M	16	W	1Y	88.2	14	Y	Y	Y	N	N	Y	N	2
D107	F	32	H	1Y	199	65.3	Y	Y	Y	N	Y	Y	N	3
D114	F	28	W	1Y	117	22.4	Y	Y	Y	N	N	N	N	1

*AA, African American; A, Asian; H, Hispanic; W, white.

[#]AE, angioedema; UR, upper respiratory; LR, lower respiratory; GI, gastrointestinal; ↓BP, decreased blood pressure.[‡]D70 and D70B are not related.

Table S2A. Ara h 2. Intensities for sera #1-5

Peptide	Sequence	Intensity									
		D19		D44		D48		D50		D53	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	RQQWELQGD RRCQSQLERAN	1,825	69	5,192	658	8,631	390	-266	68	-325	35
2	WELQGD RRCQSQLERANLRP	2,226	146	3,218	998	5,541	1,503	3	48	-298	58
3	QGD RRCQSQLERANLRPCEQ	1,691	2	2,725	88	5,555	653	-319	81	-310	93
4	RRCQSQLERANLRPCEQHLM	2,131	101	2,845	193	4,951	21	-322	112	-298	117
5	QSQLERAN LRPCEQHLMQKI	-253	99	-27	18	-38	144	1,248	327	-305	81
6	LERAN LRPCEQHLMQKIQRD	40	23	466	64	5	249	356	128	-356	45
7	AN LRPCEQHLMQKIQRDEDS	-184	87	-264	112	22	321	67	232	-309	58
8	LRPCEQHLMQKIQRDEDSYER	-285	115	-3	154	0	310	-189	8	-55	224
9	EQHLMQKIQRDEDSYERDPY	-301	136	-237	135	68	331	-296	78	-320	80
10	LMQKIQRDEDSYER DPYSPS	-292	152	88	100	4,599	1,466	-27	70	-199	9
11	KIQRDEDSYER DPYSPSQDP	-264	101	227	262	3,915	887	-286	175	-296	34
12	RDEDSYER DPYSPSQDPYSP	-191	81	-33	120	3,091	738	-156	21	-118	57
13	DSYERDPYSP SQDPYSPSQD	-214	63	-108	152	3,665	494	-197	35	-226	7
14	ER DPYSPSQDPYSPSQDPDR	-196	109	19	101	3,981	697	-109	54	-108	106
15	PYSPSQ DPYSPSQDPDRRDP	-157	60	-36	102	1,781	746	-182	137	-195	27
16	PSQ DPYSPSQDPDRRDPYSP	-278	127	92	10	3,400	1,250	385	27	544	623
17	DPYSPSQDPDRRDPYSPSPY	-127	98	390	115	4,462	1,129	761	591	148	74
18	SPSPYDRRGAGSSQHQRCC	-247	83	93	60	122	146	-215	129	-280	80
19	PYDRRGAGSSQHQRCCNEL	-256	60	-217	176	51	199	-301	93	-359	66
20	RRGAGSSQHQRCCNELNEF	-250	127	317	477	807	41	-316	141	-295	56
21	AGSSQHQRCCNELNEFENN	-302	115	1,208	429	1,099	47	-325	105	-340	85
22	SQHQRCCNELNEFENN QRC	-74	35	5,133	1,306	434	453	-241	105	182	115
23	QERCCNELNEFENN QRCMCE	424	296	9,882	1,088	1,859	252	1,834	864	1,358	482
24	CCNELNEFENN QRCMCEALQ	-162	42	5,210	95	739	210	235	477	431	373
25	ELNEFENNQRCMCEALQ QIM	157	166	8,281	2,048	118	121	-325	128	379	52
26	EFENNQRCMCEALQ QIMENQ	-298	148	35	38	-203	139	-340	127	-316	105
27	NNQRCM CEALQQIMENQSDR	-288	136	-222	97	1,438	361	-278	91	-364	54
28	RCM CEALQQIMENQSDRLQG	-283	124	-248	80	6,255	917	3,824	878	-323	87
29	CEALQQIMENQSDRLQGRQQ	897	111	505	38	4,402	332	4,432	380	-304	93
30	LQQIMENQSDRL QGRQQEQQ	-319	89	-179	58	3,048	198	2,960	385	-351	77
31	IMENQSDRL QGRQQEQQFKR	-261	187	6,216	365	414	63	2,645	338	-314	45
32	NQSDRL QGRQQEQQFKRELRL	-317	120	3,250	192	82	57	748	12	-307	71
33	DRLQGRQQEQQFKRELRLNP	7,584	40	1,895	155	1,051	54	18,027	4,738	-48	44
34	QGRQQEQQ FKRELRLNPQQC	4,873	416	-74	48	945	22	15,152	4,251	-145	64
35	QQEQQ FKRELRLNPQQCGLR	6,229	438	-158	49	779	91	16,138	5,096	-35	109
36	QQ FKRELRLNPQQCGLRAPQ	5,466	223	572	195	656	52	15,202	3,878	-68	163
37	KRELRLNPQQCGLRAPQRCD	4,213	731	603	94	1,627	143	14,481	3,106	-263	25
38	LRNLP QQCGLRAPQRCDLDV	1,183	85	5,559	303	3,327	330	6,653	140	-316	112
39	LP QQCGLRAPQRCDLVESG	470	98	3,222	14	2,066	476	3,769	14	-302	111
40	QCGLRAPQRCDLVESGGRD	-140	71	1,988	59	989	208	2,479	318	-311	53
41	LRAPQRCDLVESGGRDRY	-277	128	2,038	275	99	336	523	184	-290	83
nAra h 2	Native Ara h 2	2,168	147	3,066	339	6,199	1,525	9,971	4,062	11,372	1,428

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S2B. Ara h 2. Intensities for sera #6-10

		Intensity									
	Sera:	D59		D60		D61		D63		D64	
Peptide	Sequence	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	RQQWELQGD RRCQSQLER AN	4,291	1,846	2,948	37	3,827	362	-168	76	-295	187
2	WELQGD RRCQSQLER ANLRP	3,526	2,329	2,197	114	2,601	540	75	76	-272	232
3	QGD RRCQSQLER ANLRPCEQ	1,199	380	963	346	1,650	603	4	95	-366	209
4	RRCQSQLER ANLRPCEQHLM	4,720	2,250	2,921	300	2,345	353	502	15	-129	21
5	QSQLERAN LPCEQHLMQKI	-171	71	-270	37	-276	122	-207	39	2,611	718
6	LERAN LPCEQHLMQKI QRD	-309	18	-266	26	-304	117	-269	60	2,242	1,229
7	AN LPCEQHLMQKI QRDEDS	-228	87	168	478	1,032	1,015	-243	89	2,254	1,404
8	RPCEQHLMQKI QRDEDSYER	-312	21	-237	35	-194	151	-263	57	2,301	293
9	EQHLMQKIQRDEDSYERDPY	-307	43	-194	68	-133	238	-222	15	4,240	161
10	LMQKIQRDEDSYER DPYSPS	4,934	3,456	-72	202	5,964	1,248	19,132	7,829	16,485	5,634
11	KIQRDEDSYER DPYSPSQDP	3,164	1,677	-190	88	3,413	726	11,446	1,797	9,181	35
12	RDEDSYER DPYSPSQDPYSP	2,453	2,105	-10	146	3,881	1,008	11,575	3,796	11,579	5,211
13	DSYER DPYSPSQDPYSPSQD	5,003	3,372	-68	208	3,115	1,096	9,762	2,110	12,314	5,174
14	ER DPYSPSQDPYSPSQDPDR	6,623	4,796	-59	176	4,049	1,211	15,277	5,505	17,301	6,636
15	PYSPSQ DPYSPSQDPDRRDP	6,915	4,296	-217	52	657	207	5,884	810	14,069	2,394
16	PSQ DPYSPSQDPDRRDPYSP	6,170	3,131	-263	49	2,317	564	17,002	4,325	16,314	1,532
17	DPYSPSQDPDRRDPYSPSPY	8,092	5,916	0	235	4,566	122	22,714	12,053	13,300	3,589
18	SPSPYDRRGAGSSQHQRCC	-234	33	-264	30	-157	13	-208	57	-156	100
19	PYDRRGAGSSQHQRCCNEL	-284	31	-273	1	-254	74	-284	85	-156	15
20	RRGAGSSQHQRCCNELNEF	-273	23	-258	37	-273	100	-243	39	-134	156
21	AGSSQHQRCCNELNEFENN	-274	28	-239	37	-214	25	-269	81	-255	115
22	SQHQRCC NELNEFENNQR C	-273	11	-248	3	-304	127	-221	101	-255	148
23	QERCC NELNEFENNQR CMCE	-298	2	-260	41	-307	103	181	173	-367	219
24	CNELNEFENNQR CMCEALQ	-278	9	-291	22	-302	116	-194	138	-336	151
25	ELNEFENNQR CMCEALQ QIM	-269	21	-280	19	-305	126	-25	60	-117	83
26	EFENNQR CMCEALQ QIMENQ	-273	31	-286	28	-315	129	-242	54	-266	124
27	NNQR CMCEALQQIMENQ SDR	-272	31	-263	29	970	343	-268	63	7,398	1,128
28	RCM CEALQQIMENQ SDRLQG	-273	2	-278	24	3,836	646	108	26	7,264	10
29	CEALQQIMENQ SDRLQGRQQ	-228	52	542	64	2,769	890	189	28	6,002	1,003
30	LQQIMENQSDR LQGRQQEQ EQ	-238	14	890	332	2,340	767	-95	37	5,294	1,833
31	IMENQSDR LQGRQQEQ EQFKR	-187	126	5,067	1,481	900	716	217	100	1,069	244
32	NQSDR LQGRQQEQ EQFKREL	-288	27	571	378	10	231	243	97	249	332
33	DR LQGRQQEQ EQFKRELRLNP	-227	6	2,380	103	1,352	504	1,329	200	15,861	6,645
34	QGRQQEQ QFKRELRLNP QQC	-318	21	1,837	244	1,169	475	1,204	530	12,412	4,582
35	QQEQ QFKRELRLNP QQCGLR	-260	13	1,659	237	1,285	505	1,762	610	14,763	3,579
36	QQFKRELRLNP QQCGLRAPQ	77	24	1,263	250	1,647	213	-19	18	14,252	3,478
37	KRELRLNP QQCGLRAPQRCD	-288	26	1,070	322	1,099	369	-188	32	12,386	3,616
38	LRNL PQQCGLRAPQRCD LDV	126	401	-212	52	-165	96	-211	91	4,453	1,441
39	LP QQCGLRAPQRCD LDVESG	-267	22	-247	39	-226	15	-169	4	3,804	593
40	QCGLRAPQRCD LDVESGGRD	-267	5	-274	29	-133	6	-188	5	2,869	856
41	LRAPQRCDLDVESGGRDRY	-267	52	-281	23	-250	49	-257	96	18	71
nAra h 2	Native Ara h 2	8,707	3,266	13,430	3,780	8,463	477	23,982	15,596	21,492	10,754

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S2C Ara h 2. Intensities for sera #11-15

		Intensity									
	Sera:	D65		D66		D67		D68		D69	
Peptide	Sequence	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	RQQWELQGD RRCQSQLERAN	7,518	158	15,392	3,863	19,775	3,879	1,081	342	8,711	86
2	WELQGD RRCQSQLERANLRP	5,948	593	16,443	7,431	14,547	3,740	918	117	5,230	1,459
3	QGD RRCQSQLERANLRPCEQ	2,800	238	9,167	2,360	10,796	2,583	729	78	6,362	407
4	RRCQSQLERANLRPCEQHLM	4,430	875	22,602	12,026	22,250	8,626	1,667	472	9,216	1,457
5	QSQLERAN LPCEQHLMQKI	-483	64	-710	134	132	282	21	159	-155	68
6	LERAN LPCEQHLMQKIQRD	-597	80	-893	223	-763	386	-395	204	-242	36
7	AN LPCEQHLMQKIQRDEDS	-593	89	-733	320	-872	488	314	432	-131	30
8	RPCEQHLMQKIQRDEDSYER	-374	309	-906	189	-818	450	-242	86	-265	4
9	EQHLMQKIQRDEDSYERDPY	-513	144	-891	188	-829	457	-316	195	-285	71
10	LMQKIQRDEDSYER DPYSPS	-532	90	1,504	371	8,985	1,228	-207	294	430	677
11	KIQRDEDSYER DPYSPSQDP	-547	56	743	312	8,980	585	-340	226	94	341
12	RDEDSYER DPYSPSQDPYSP	353	168	2,184	21	14,695	3,484	-203	310	127	376
13	DSYER DPYSPSQDPYSPSQD	42	52	2,184	350	13,217	1,911	-225	250	51	286
14	ER DPYSPSQDPYSPSQDPDR	-296	87	3,263	341	14,138	3,775	-311	260	252	307
15	PYSPSQ DPYSPSQDPDRRDP	-523	57	44	197	9,299	1,452	-297	246	343	394
16	PSQ DPYSPSQDPDRRDPYSP	-437	100	2,666	228	7,917	2,268	-393	255	64	290
17	DPYSPSQDPDRRDPYSPSPY	2,079	634	5,279	723	9,158	3,576	-190	115	330	459
18	SPSPYDRRGAGSSQHQRCC	200	0	-914	170	-712	318	-339	257	-61	221
19	PYDRRGAGSSQHQRCCNEL	-542	110	-856	139	-743	373	-371	240	-264	1
20	RRGAGSSQHQRCCNELNEF	-511	139	-739	89	-285	348	-414	212	-314	59
21	AGSSQHQRCCNELNEFENN	-548	135	-869	173	-818	496	-409	199	-294	50
22	SQHQRCC NELNEFENNQR C	200	325	-901	194	-232	1,007	-289	178	-246	57
23	QERCC NELNEFENNQR CMCE	331	65	-952	210	4,764	1,752	-419	211	-300	35
24	CNELNEFENNQR CMCEALQ	-186	157	-948	190	62	1,136	-390	183	-227	13
25	ELNEFENNQR CMCEALQ QIM	-396	117	-912	192	172	835	-388	185	-298	56
26	EFENNQR CMCEALQ QIMENQ	-566	99	-919	162	-751	535	-385	205	-313	32
27	NNQR CMCEALQ QIMENQSDR	-524	144	-869	156	-777	381	-317	218	-287	39
28	RCM CEALQ QIMENQSDRLQG	-401	141	-796	79	-504	267	-132	293	5,633	820
29	CEALQ QIMENQSDRLQGRQQ	883	489	-803	142	-223	201	-186	133	4,952	1,380
30	LQQIMENQSDRL QGRQQEQQ	793	173	-865	121	-374	368	-338	182	2,396	6
31	IMENQSDRL QGRQQEQQ FKR	4,220	37	-559	135	-817	446	-255	115	2,178	71
32	NQSDRL QGRQQEQQ FKRELRL	1,283	588	-662	230	-885	481	-326	140	379	59
33	DR QGRQQEQQ FKRELRLNP	1,107	746	-646	249	5,165	592	-364	177	-168	108
34	QGRQQEQQ FKRELRLNP QQC	-498	138	-623	158	3,698	214	-337	169	-158	77
35	QQEQQ FKRELRLNP QCGLR	-552	148	-612	211	4,238	142	-303	243	-201	98
36	QQ FKRELRLNP QCGLRAPQ	-551	127	-690	112	3,381	178	-244	307	87	161
37	KRELRLNP QCGLRAPQRCD	-534	175	-636	163	3,915	742	-371	196	-221	89
38	LRNL PQCGLRAPQRCD LDV	-300	125	-715	289	6,539	1,482	-334	265	-302	52
39	LPQ QCGLRAPQRCD LDVESG	-484	128	-851	214	3,458	166	-338	268	-192	91
40	QCGLRAPQRCD LDV ESG GRD	-579	116	-931	228	930	839	-371	237	-150	115
41	LRAPQRCDLDV ESG GRDRY	-524	130	-845	104	-720	412	-377	233	-279	32
nAra h 2	Native Ara h 2	9,364	844	11,851	2,815	14,289	6,355	3,135	829	17,073	11,168

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S2D. Ara h 2. Intensities for sera #16-20

Peptide	Sequence	Intensity									
		D70		D70B		D71		D72		D74	
		Mean	SEM	Mean*	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	RQQWELQGD RRCQSQLER AN	7,617	28	6,583	491	6,274	3,233	-477	276	3,945	186
2	WELQGD RRCQSQLER ANLRP	4,818	685	7,554	1,392	5,151	747	-435	275	3,451	699
3	QGD RRCQSQLER ANLRPCEQ	3,745	38	4,640	499	3,899	1,902	-533	254	2,434	1,443
4	RRCQSQLER ANLRPCEQHLM	5,206	786	9,038	3,183	7,149	1,146	-460	239	4,845	255
5	QSQLERANL RPCEQHLMQK I	-236	46	337	100	497	3	-469	221	-189	146
6	LERANL RPCEQHLMQK IQRD	-246	38	-130	51	434	451	-475	272	542	565
7	ANL RPCEQHLMQK IQRDEDS	-229	18	-250	203	150	553	-529	265	-212	177
8	RPCEQHLMQK IQRDEDSYER	-223	30	-233	141	104	321	-525	306	321	49
9	EQHLMQKIQRDEDSYERDPY	-214	15	-244	170	73	345	-538	309	-226	179
10	LMQKIQRDEDSYER DPYSPS	5,830	827	6,539	3,138	1,003	799	-414	277	841	576
11	KIQRDEDSYER DPYSPS QDP	1,774	66	2,251	742	9,822	3,883	-301	281	1,065	699
12	RDEDSYER DPYSPS QDPYSP	3,379	744	6,823	1,901	18,552	8,666	-94	236	508	253
13	DSYER DPYSPS Q DPYSPS QD	2,697	749	5,711	1,532	16,350	7,514	145	419	453	305
14	ERDPYSPS QDPYSPS QDPDR	2,461	213	6,800	2,231	22,271	10,606	130	363	914	712
15	PYSPS QDPYSPS QDPDRRDP	154	115	2,593	1,544	18,349	8,238	-161	396	1,269	733
16	PS QDPYSPS QDPDRRDPYSP	1,028	333	7,036	3,054	10,634	4,135	-370	252	546	451
17	DPYSPS QDPDRRDPYSPSPY	4,324	779	11,409	3,857	5,369	1,577	-125	67	882	588
18	SPSPYDRRGAGSSQHQRCC	-199	36	-116	64	54	158	-433	245	20	346
19	PYDRRGAGSSQHQRCCNEL	-190	40	-203	105	-282	122	-478	214	-220	130
20	RRGAGSSQHQRCCNELNEF	1,253	711	-159	126	-6	12	-531	240	-187	193
21	AGSSQHQRCCNELNEFENN	-153	62	573	312	2,154	249	-559	267	-204	194
22	SQHQRCCNELNEFENN QRC	-107	27	1,492	234	1,889	539	-521	248	-127	228
23	QERCCNELNEFENN QRC MCE	922	518	5,662	1,382	7,995	404	-502	251	-214	124
24	CCNELNEFENN QRC MCEALQ	-145	57	2,479	200	2,095	8	-507	213	-260	144
25	ELNEFENN QRC MCEALQQIM	-262	57	5,648	3,374	6,006	2,188	-432	153	-225	176
26	EFENN QRC MCEALQQIMENQ	-226	23	-201	97	180	109	-537	288	-227	161
27	NN QRC MCEALQQIMEN QSDR	-252	28	2,372	462	6,218	1,476	-514	213	-208	162
28	RCMCEALQQIMEN QSDR LQG	-245	37	4,984	581	15,986	2,493	-493	206	-162	146
29	CEALQQIMEN QSDR LQGRQQ	-239	28	4,405	470	10,947	3,704	-396	180	947	412
30	LQQIMEN QSDR LQGRQQEQQ	-232	10	2,658	168	4,540	447	-540	296	-190	146
31	IMEN QSDR LQGRQQEQQFKR	560	332	968	183	7,387	3,200	-465	265	-50	166
32	NQSDRLQGRQQEQQFKRELRL	-190	12	197	61	4,460	2,405	-453	356	-200	117
33	DR LQGRQQEQQ FKRELRLNP	6,931	1,132	4,628	15	11,382	413	-472	288	-86	83
34	QGRQQEQQFKRELRLNP QQC	6,301	99	3,352	262	6,775	564	-419	148	-133	170
35	QQEQQFKRELRLNP QQC GLR	5,892	424	3,718	113	8,341	293	238	294	-208	129
36	QQFKRELRLNP QQC GLRAPQ	7,804	295	3,581	8	8,898	231	-176	32	1,727	1,002
37	KRELRLNPQQC GLRAPQRCD	5,969	205	2,708	170	6,219	394	-467	194	165	227
38	LRNLP QQCGLRAPQRCD LDV	-93	74	2,889	161	11,658	395	-450	173	-232	139
39	LP QQCGLRAPQRCD LDVESG	-216	36	1,993	318	8,535	154	-556	267	-170	133
40	QCGLRAPQRCD LDVESGGRD	-232	22	1,162	127	5,061	146	-577	264	62	190
41	LRAPQRCDLDVESGGRDRY	-135	49	234	2	2,324	32	-429	125	-184	137
nAra h 2	Native Ara h 2	8,865	2,064	19,313	7,543	18,950	9,950	5,186	1,958	7,677	3,173

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S2E. Ara h 2. Intensities for sera #21-25

Peptide	Sera:	Intensity									
		D77		D78		D80		D81		D82	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	RQQWELQGD RRCQSQLERAN	6,400	1,752	9,099	571	-177	77	10,092	1,161	9,687	153
2	WELQGD RRCQSQLERANLRP	3,254	59	6,436	643	261	392	15,035	2,295	7,721	288
3	QGD RRCQSQLERANLRPCEQ	3,833	1,203	4,909	520	-250	163	8,743	1,007	6,688	521
4	RRCQSQLERANLRPCEQHLM	6,301	1,426	5,230	693	-225	91	15,487	28	13,086	861
5	QSQLERANL RPCEQHLMQKI	27	250	-70	19	-151	73	537	402	-21	349
6	LERANL RPCEQHLMQKIQRD	-239	64	-145	12	-228	141	-48	354	-164	248
7	ANL RPCEQHLMQKIQRDEDS	-216	11	-134	32	-262	107	2,563	967	3,181	709
8	RPCEQHLMQKIQRDEDSYER	-273	42	-138	4	-258	132	511	158	185	181
9	EQHLMQKIQRDEDSYERDPY	674	631	-144	28	-256	124	856	663	394	303
10	LMQKIQRDEDSYER DPYSPS	1,840	558	-50	50	186	508	2,859	899	-21	255
11	KIQRDEDSYER DPYSPSQDP	2,449	1,401	7	22	112	391	1,135	682	4,308	424
12	RDEDSYER DPYSPSQDPYSP	4,191	1,863	1,675	135	3,361	1,583	4,560	1,005	11,244	319
13	DSYER DPYSPSQDPYSPSQD	3,537	1,624	444	86	1,571	22	3,414	974	9,765	682
14	ER DPYSPSQDPYSPSQDPDR	2,531	1,177	329	6	2,646	2,072	5,656	1,822	11,447	2,391
15	PYSPSQ DPYSPSQDPDRRDP	2,501	1,245	157	6	835	11	2,302	1,063	4,699	238
16	PSQ DPYSPSQDPDRRDPYSP	1,095	474	1,186	107	1,101	1,435	3,243	1,438	571	15
17	DPYSPSQDPDRRDPYSPSPY	2,030	576	976	250	806	717	6,922	2,448	278	249
18	SPSPYDRRGAGSSQH QERCC	-197	46	-88	25	-142	157	-235	273	-295	151
19	PYDRRGAGSSQH QERCCNEL	-275	62	-134	19	-181	72	-266	191	-293	168
20	RRGAGSSQH QERCCNELNEF	-82	171	-143	13	-209	73	428	527	-335	137
21	AGSSQH QERCCNELNEFENN	-210	54	-134	9	-175	53	-298	181	-299	183
22	SQH QERCCNELNEFENNQR C	-214	32	-91	6	-251	205	-159	340	-280	194
23	QER CCNELNEFENNQR CMCE	-288	11	6	27	-215	248	-208	121	-188	266
24	CCNELNEFENNQR CMCEALQ	-227	6	-109	21	-268	176	-241	269	-46	443
25	ELNEFENNQR CMCEALQQIM	141	175	-77	13	-125	56	-254	293	51	413
26	EFENNQR CMCEALQQIMENQ	-167	33	-163	18	-217	95	-209	298	-282	163
27	NNQR CMCEALQQIMENQSDR	-253	6	-167	20	-274	154	-228	273	-276	151
28	RCM CEALQQIMENQSDRLQG	-300	27	-93	38	-208	48	-28	40	-263	155
29	CEALQQIMENQSDRLQGRQQ	-57	157	-5	23	-18	73	419	112	-267	224
30	LQQIMENQSDRL QGRQQEQQ	-145	11	40	73	-192	33	-13	134	-168	150
31	IMENQSDRL QGRQQEQQFKR	2,093	1,305	4,676	138	90	162	451	298	-228	247
32	NQSDRL QGRQQEQQFKREL R	818	688	3,499	57	326	551	226	177	-341	170
33	DR QGRQQEQQFKREL RNL	431	325	3,075	30	170	199	1,946	566	174	176
34	QGRQQEQQ FKREL RNL PQQ C	-153	91	-109	54	-203	110	1,338	185	-42	223
35	QQEQQ FKREL RNL PQQ CGLR	-192	103	-155	10	-117	163	957	134	-48	198
36	QQ FKREL RNL PQQ CGLRAPQ	-135	23	-125	29	-17	281	759	567	198	533
37	KREL RNL PQQ CGLRAPQRCD	-275	21	-129	23	128	433	458	269	-25	125
38	LRNL PQQCGL RAPQRCDL DV	-278	52	-179	5	586	815	-101	30	-343	140
39	LP QQCGL RAPQRCDL DV ESG	-233	103	-187	6	-217	73	-330	205	-327	157
40	QCGL RAPQRCDL DV ESGGRD	-117	57	53	206	-125	17	-262	209	-339	151
41	LRAPQRCDL DVESGGR DRY	-156	23	-162	22	-199	95	397	416	-320	145
nAra h 2	Native Ara h 2	10,347	4,402	8,283	1,744	15,323	528	14,790	3,504	7,799	2,176

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S2F. Ara h 2. Intensities for sera #26-30

Peptide	Sequence	Intensity									
		D98		D103		D105		D107		D114	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean*	SEM	Mean	SEM
1	RQQWELQGD RRCQSQLERAN	2,060	151	10,472	4,579	1,943	0	12,427	653	5,115	233
2	WELQGD RRCQSQLERANLRP	458	262	9,941	2,703	803	380	9,621	663	6,388	199
3	QGD RRCQSQLERANLRPCEQ	386	227	6,822	3,125	1,193	663	6,914	1,737	5,485	14
4	RRCQSQLERANLRPCEQHLM	1,654	189	13,279	1,103	703	156	11,865	2,703	7,388	299
5	QSQLERAN LRPCEQHLMQKI	557	756	-120	32	-470	83	-96	36	-335	261
6	LERAN LRPCEQHLMQKIQRD	-175	260	-315	90	-403	75	-308	218	-245	283
7	AN LRPCEQHLMQKIQRDEDS	-222	86	3,542	2,715	-419	52	-375	181	383	528
8	LRPCEQHLMQKIQRDEDSYER	-469	312	769	701	-497	72	-400	183	-323	214
9	EQHLMQKIQRDEDSYERDPY	-274	147	1,317	1,515	-473	134	-411	153	-362	267
10	LMQKIQRDEDSYER DPYSPS	-349	299	13,029	5,384	-486	96	167	206	-430	217
11	KIQRDEDSYER DPYSPS QDP	730	711	7,631	4,975	-535	119	310	212	-436	235
12	RDEDSYER DPYSPS QDPYSP	-67	372	6,199	4,106	-485	50	1,087	390	-334	255
13	DSYERDPYSP SQDPYSPS QD	-354	317	6,821	4,248	-465	21	421	291	-329	286
14	ER DPYSPS QDPYSP SQDPDR	-23	274	8,655	6,272	-375	180	1,608	665	-224	359
15	PYSP SQDPYSPS QDPDRRDP	-147	405	530	323	-316	51	1,356	300	-428	264
16	PSQ DPYSPS QDPDRRDPYSP	-234	383	3,260	2,628	-495	44	678	181	-410	225
17	DPYSPS QDPDRRDPYSPSPY	-303	378	12,548	6,710	-348	148	959	141	-303	221
18	SPSPYDRRGAGSSQHQRCC	-429	307	-281	136	-266	67	-372	168	-305	120
19	PYDRRGAGSSQHQRCCNEL	-427	305	-287	132	-386	49	-378	118	-427	194
20	RRGAGSSQHQRCCNELNEF	-421	276	87	102	-520	110	-370	127	-411	217
21	AGSSQHQRCCNELNEFENN	-497	328	-307	71	-556	106	-136	117	-374	129
22	SQHQRCCNELNEFENN QRC	-100	127	-304	35	167	447	-171	81	-292	228
23	QERCCNELNEFENN QRC MCCE	-419	215	-165	5	996	250	-156	235	598	453
24	CCNELNEFENN QRC MCCEALQ	-478	285	-322	71	-111	178	-262	211	-200	317
25	ELNEFENNQRCMCCEALQQIM	278	474	-307	91	-271	68	-137	18	-139	356
26	EFENNQRCMCCEALQQIMENQ	430	638	-375	54	-544	30	-464	214	-468	227
27	NNQRCMC CEALQQIMENQSDR	437	641	-347	111	-388	146	-400	238	65	383
28	RCMC CEALQQIMENQSDR LQGG	-437	289	-288	143	-127	448	-306	122	198	416
29	CEALQQIMENQSDR LQGRQQ	-358	276	3,234	219	281	166	8,825	829	-160	279
30	LQQIMENQSDR LQGRQQEQQ	-394	303	1,464	18	-200	218	5,479	1,483	-238	264
31	IMENQSDR LQGRQQEQQ FKR	-427	227	2,014	293	5	120	5,718	1,283	-400	235
32	NQSDR LQGRQQEQQ FKRELRL	-387	216	431	163	-281	235	1,997	646	-397	190
33	DRL LQGRQQEQQ FKRELRLNP	462	221	-154	166	-232	228	1,213	153	-342	226
34	QGRQQEQQ FKRELRLNPQQC	180	136	-230	127	-351	129	-196	259	-398	261
35	QQEQQ FKRELRLNPQQC GLRL	-7	216	3	333	-188	395	-67	272	-399	222
36	QQ FKRELRLNPQQC GLRAPQ	85	462	817	881	100	156	-64	268	-421	226
37	FKRELRLNPQQC GLRAPQRCD	174	115	-197	123	-331	103	-144	212	-338	274
38	LRNLP QQCGLRAPQRCDLDV	-108	280	-308	107	-418	184	-57	117	266	463
39	LP QQCGLRAPQRCDLDV ESG	74	272	-319	53	-489	134	-6	3	-209	256
40	QCGLRAPQRCDLDV ESGGRD	-309	296	-312	49	-462	39	-280	80	-336	223
41	LRAPQRCDLDVESGGRDRY	-479	317	-296	91	-445	147	-217	49	-426	201
nAra h 2	Native Ara h 2	8,698	139	15,410	2,251	1,785	22	8,082	1,723	1,832	42

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots (n=18-21) + 4 SD of the empty spots)

Table S3A. Ara h 6. Intensities for sera #1-5

Peptide	Sequence	Intensity									
		D19		D44		D48		D50		D53	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	MRRERG RQGDSSS CERQVDR	-327	127	5,992	1,636	-181	150	-304	104	-278	62
2	ERG RQGDSSS CERQVDRVNL	-322	137	-318	95	-37	93	-312	105	-310	115
3	RQGDSSS CERQVDRVNLKPC	521	508	677	709	-45	216	-165	14	-318	110
4	DSSCERQVDRVNLKPCEQH	-275	124	-209	105	36	95	-171	91	-309	81
5	SCERQVDRVNLKPCEQIMQ	-260	97	-264	23	149	194	-171	30	-303	99
6	RQVDRVNL KPCEQHIMQR IM	-301	124	-274	57	-80	139	647	822	-8	386
7	DRVNL KPCEQHIMQR IMGEQ	-159	7	-163	25	83	235	-206	37	-306	122
8	NL KPCEQHIMQR IMGEQEY	-314	100	-288	69	-99	128	-293	92	-314	90
9	PCEQHIMQRIMGEQEYDSY	-325	127	-287	9	-180	197	-165	64	-325	101
10	QHIMQRIMGEQEY YDSYDIR	-59	135	-243	26	325	238	-220	14	2,848	1,055
11	MQRIMGEQEY YDSYDIR STR	-334	133	-257	21	21	243	-202	33	705	226
12	IMGEQEY YDSYDIR STRSSD	-314	144	-287	81	226	180	-311	106	1,229	221
13	EEQEY YDSYDIR STRSSDQQ	-305	129	-244	30	-33	243	-241	54	254	19
14	QYDSYDIR STRSSDQQQRCC	-199	95	88	86	56	129	-152	25	193	222
15	SYDIRSTRSSDQQQRCCDEL	-303	140	-344	103	-170	156	-331	110	-330	98
16	IRSTRSSDQQQRCCDELNEM	-294	101	-320	120	-111	170	-340	124	-302	62
17	TRSSDQQQRCCDELNEMENT	-290	96	-310	107	-118	170	-352	117	-326	96
18	SDQQQRCCDELNEMENT QRC	-8	29	5,513	321	272	16	-339	133	-311	103
19	QQRC CDELNEMENTQRC MCCE	-280	81	1,787	704	-117	139	-288	105	-313	115
20	CDELNEMENTQRC MCCEALQ	-261	99	1,429	28	-149	184	-312	127	-303	86
21	ELNEMENTQRCMCCEALQIM	-259	109	244	290	-156	119	149	516	-283	51
22	EMENTQRCMCCEALQIMENQ	-306	124	-313	51	-94	43	-335	120	-304	98
23	NTQRCM CEALQQIMENQCD R	-296	95	-264	131	1,401	25	-317	106	-315	112
24	RCM CEALQQIMENQCD RLQD	-272	105	-282	106	712	114	-343	129	-269	158
25	CEALQQIMENQCD RLQDRQM	-202	128	-4	211	1,576	266	-17	128	-307	85
26	LQQIMENQCDRLQDRQMVQQ	-297	139	-266	54	160	31	-305	85	-281	104
27	IMENQCDRLQDRQMVQQFKR	-265	116	-100	28	145	186	-91	97	-271	101
28	NQCDRLQDRQMVQQFKRELM	-280	154	-111	11	827	144	-336	95	-302	120
29	DRLQDRQMVQQFKRELMNLP	14,269	1,138	115	210	1,492	410	28,276	5,188	-218	185
30	QDRQMVQQF KRELMNLPQQC	1,925	251	-21	107	412	438	12,722	7,688	-317	83
31	QMVQQF KRELMNLPQQC NFR	2,017	351	-129	114	361	402	8,381	5,841	-255	165
32	QQF KRELMNLPQQC NFRAPQ	1,940	330	483	16	445	419	7,547	4,892	-312	116
33	KRELMNLPQQC NFRAPQRCD	782	110	1,260	218	1,241	23	14,289	6,280	-297	127
34	LMNLPQQ CNFRAPQRCDLDV	72	11	3,665	640	2,834	185	5,582	556	-326	99
35	LPQQ CNFRAPQRCDLDV SGG	-158	67	1,949	3	943	372	1,660	45	-316	106
36	QCNFRAPQRCDLDV SGGRC	-240	94	1,869	238	182	421	342	301	-292	89
nAra h 6	Native Ara h 6	5,950	175	5,850	61	5,896	1,884	7,574	92	5,372	2,579

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S3B. Ara h 6: Intensities for sera #6-10

		Intensity									
Sera:		D59		D60		D61		D63		D64	
Peptide	Sequence	Mean	SEM	Mean	Mean	Mean	SEM	Mean	SEM	Mean	SEM
1	MRRERG RQGDSSS CERQVDR	-276	21	-268	35	-282	81	-66	231	-265	93
2	ERG RQGDSSS CERQVDRVNL	-300	22	-253	15	-234	37	-255	87	-296	112
3	RQGDSSS CERQVDRVNLKPC	-45	166	-282	16	-220	6	139	182	-262	100
4	DSSSCERQVDRVNLKPCEQH	-105	123	-262	39	-122	74	-155	30	-240	97
5	SCERQVDRVNLKPCEQHIMQ	-202	33	-225	11	-267	107	-223	40	-232	69
6	RQVDRVNL KPCEQHIMQR IM	127	200	-246	25	-245	84	-73	11	928	359
7	DRVNL KPCEQHIMQR IMGEQ	-259	30	-225	16	-286	109	-195	3	6,644	592
8	NL KPCEQHIMQR IMGEQEQY	-274	21	-258	18	-314	122	-246	67	1,312	28
9	PCEQHIMQRIMGEQEQYDSY	-306	11	-263	2	-301	124	-240	37	321	421
10	QHIMQRIMGEQEQ YDSYDIR	-233	24	-259	38	-291	149	-231	60	-189	32
11	MQRIMGEQEQ YDSYDIR STR	-160	141	-295	35	-174	242	-241	77	-324	154
12	IMGEQEQ YDSYDIR STRSSD	-308	9	-137	39	-288	104	-280	75	-319	134
13	EEQEQ YDSYDIR STRSSDQQQ	-300	35	-274	16	-334	110	-282	71	-294	139
14	YDSYDIR STRSSDQQQRCC	-272	8	-274	24	-264	97	-200	75	-256	141
15	SYDIRSTRSSDQQQRCCDEL	-312	11	-229	30	-280	110	-267	61	-302	205
16	IRSTRSSDQQQRCCDELNEM	-285	8	-233	29	-310	141	-286	68	-360	183
17	TRSSDQQQRCCDELNEMENT	-308	8	-240	24	-305	95	-269	37	-335	157
18	SDQQQRCCDELNEMENT QRC	-264	21	-260	40	-310	101	-181	101	-294	149
19	QQRC DELNEMENTQRC MCCE	-287	14	-2	95	-293	110	-260	116	-299	180
20	DELNEMENTQRC MCCEALQ	-271	58	-286	22	-259	83	-250	60	-277	103
21	ELNEMENT QRC MCCEALQQIM	-289	33	-266	28	-281	95	-250	36	-247	109
22	EMENT QRC MCCEALQQIMENQ	-305	35	-300	22	-284	89	-286	71	-232	82
23	NT QRC MC CEALQQIMENQ CDR	-253	11	137	16	1,542	535	-249	91	4,816	551
24	RCM CEALQQIMENQ CDRLQD	-248	3	-292	17	1,395	549	-272	62	2,834	293
25	CEALQQIMENQ CDRLQDRQM	-226	17	-270	23	3,070	948	-63	139	5,146	990
26	LQQIMENQCDRLQDRQMVQQ	-251	33	-274	33	958	482	-256	80	1,279	694
27	IMENQCDRLQDRQMVQQFKR	-228	64	-246	23	-5	233	-170	37	-119	64
28	NQCDRLQDRQMVQQFKRELM	-317	16	-295	20	-315	98	-249	23	-260	145
29	DRLQDRQMVQQFKRELMNLP	42	286	993	409	1,814	194	1,406	120	27,254	9,376
30	QDRQMVQQF KRELMNLPQQ C	-312	11	-271	4	38	28	541	152	16,730	8,392
31	QMVQQF KRELMNLPQQ CNFR	-298	4	-28	138	341	96	755	199	12,874	4,884
32	QQF KRELMNLPQQ CNFRAPQ	-176	115	-199	18	326	103	-94	35	14,854	7,473
33	KRELMNLPQQ CNFRAPQRCD	-214	74	258	80	434	1	-73	4	18,439	11,221
34	LMNLPQQ CNFRAPQRCD LDV	889	931	-254	15	-313	94	-224	77	4,414	942
35	LPQQ CNFRAPQRCD LDVSGG	-328	25	-266	31	-292	107	-242	54	2,540	614
36	QCNFRAPQRCD LDVSGGRC	-278	29	-250	47	-150	35	-225	76	201	752
nAra h 6	Native Ara h 6	4,257	2,458	5,529	3,719	8,777	544	5,756	1,589	6,576	2,512

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S3C. Ara h 6: Intensities for sera #11-15

Peptide	Sera:	Intensity									
		D65		D66		D67		D68		D69	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	MRRERG RQGDSSS CERQVDR	-523	127	4,123	415	-865	479	-252	378	-310	54
2	ERG RQGDSSS CERQVDRVNL	-438	192	-950	211	-828	436	-370	262	-97	40
3	RQGDSSS CERQVDRVNLKPC	-528	134	-955	180	-711	346	-329	211	64	315
4	DSSSCERQVDRVNLKPCEQH	-529	111	-879	160	-831	431	-143	402	-68	54
5	SCERQVDRVNLKPCEQHIMQ	-558	100	-898	158	-763	467	-266	314	-162	36
6	RQVDRVNL KPCEQHIMQRI M	-507	111	-812	147	-428	112	-128	4	639	472
7	DRVNL KPCEQHIMQRI MGEQ	-621	47	-835	134	-527	565	-130	419	-101	33
8	NL KPCEQHIMQRI MGEQEY	-520	90	-804	101	-814	486	-390	187	-213	62
9	PCEQHIMQRI MGEQEYDSY	-584	83	-834	156	-739	351	-356	204	-225	39
10	QHIMQRI MGEQEYDSYDIR	-566	132	10,461	3,798	-812	435	-236	36	-200	67
11	IMGEQEY QYDSYDIR STR	154	166	6,904	597	-804	413	-348	132	-128	76
12	IMGEQEY QYDSYDIR STRSSD	311	336	7,178	508	-844	455	-383	196	-118	165
13	EQEQ YDSYDIR STRSSDQQ	-276	59	4,272	508	-827	423	-369	211	-106	177
14	QYDSYDIR STRSSDQQRC	-291	76	7,227	325	-805	424	-360	225	-104	69
15	SYDIRSTRSSDQQRCDEL	-502	132	-573	48	-671	302	-386	195	-246	40
16	IRSTRSSDQQRCDELNEM	-528	132	-768	23	-592	199	-401	201	-240	39
17	TRSSDQQRCDELNEMENT	-392	192	-861	132	-671	286	-388	211	-233	45
18	SDQQRC DELNEMENTQRC	-488	133	-849	134	-408	585	-367	221	-224	29
19	QQRC DELNEMENTQRC MCE	-538	126	-899	162	-593	301	-398	206	-228	37
20	CDELNEMENTQRC MCEALQ	-619	77	-858	113	-737	345	-410	227	-233	28
21	ELNEMENTQRCMCEALQQIM	-539	113	-970	227	-644	286	-381	214	169	298
22	EMENTQRCMCEALQQIMENQ	-478	182	-686	310	-839	434	-339	259	-243	28
23	NTQRCM CEALQQIMENQCD R	-470	172	-898	259	-813	428	-273	245	-218	8
24	RCM CEALQQIMENQCD RQLQD	-501	90	-967	269	-886	513	-229	286	-237	23
25	CEALQQIMENQCD RQLQDRQM	-411	37	-889	221	86	47	822	1,084	-53	138
26	LQQIMENQCDRLQDRQMVQQ	-555	121	-921	161	-374	12	-295	122	-132	107
27	IMENQCDRLQDRQMVQQFKR	-543	81	-725	13	-749	411	-373	229	-97	45
28	NQCDRLQDRQMVQQFKRELM	-624	32	-759	23	-814	472	-381	182	-171	21
29	DRLQDRQMVQQFKRELMNLP	-568	21	-472	206	10,166	1,621	-367	232	-80	166
30	QDRQMVQQF KRELMNLPQQC	-511	109	-225	431	959	411	-261	170	-127	132
31	QMVQQF KRELMNLPQQ CNFR	-513	53	-757	88	187	316	-219	310	-84	125
32	QQF KRELMNLPQQ CNFRAPQ	-536	93	-653	257	82	99	571	827	-60	168
33	KRELMNLPQQ CNFRAPQRCD	-517	77	-793	243	1,876	1,696	1,745	1,497	-158	69
34	LMNLPQQ CNFRAPQRCDLDV	-480	124	-784	229	4,491	832	599	384	-233	43
35	LPQQ CNFRAPQRCDLDV SGG	-432	189	-867	140	657	803	76	359	-200	1
36	QCNFRAPQRCDLDV SGGRC	-415	179	-811	95	-203	878	-423	201	-178	4
nAra h 6	Native Ara h 6	10,693	2,231	18,565	873	13,667	833	7,415	1,653	4,879	127

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S3D. Ara h 6: Intensities for sera #16-20

		Intensity									
	Sera:	D70		D70B		D71		D72		D74	
Peptide	Sequence	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	MRRERGRQGDSSSCERQVDR	-244	25	-248	147	1,151	1,010	-420	399	-242	151
2	ERGQGDSSSCERQVDRVNL	-263	34	-235	187	-54	76	-543	257	-255	142
3	RQGDSSSCERQVDRVNLKPC	-246	37	-198	85	6,276	116	-81	233	1,784	1,317
4	DSSSCERQVDRVNLKPCEQH	-254	44	-217	153	3,561	648	-511	274	165	35
5	SCERQVDRVNLKPCEQHIMQ	-226	10	42	49	2,684	813	-273	39	-72	157
6	RQVDRVNLKPCEQHIMQRI	-101	75	161	224	12,149	13	569	663	94	153
7	DRVNLKPCEQHIMQRI	-231	44	195	120	13,463	507	388	554	-79	139
8	NLKPCEQHIMQRI	-237	28	-126	23	2,449	1,262	-336	159	-186	129
9	PCEQHIMQRI	-268	51	83	167	601	699	-380	141	-213	144
10	QHIMQRI	-259	35	-179	47	221	361	-314	119	-89	120
11	MQRIMGEQEQYDSYDIRSTR	-228	68	-145	14	7,500	4,307	-462	288	138	110
12	IMGEQEQYDSYDIRSTRSSD	-251	43	-264	160	8,551	5,163	-557	301	716	503
13	EQEQYDSYDIRSTRSSDQQQ	-184	16	-257	124	7,783	2,770	-532	268	85	238
14	QYDSYDIRSTRSSDQQQRCC	-159	91	-175	101	21,819	4,993	-497	236	866	513
15	SYDIRSTRSSDQQQRCCDEL	-50	153	-254	120	27,091	6,512	-460	200	-236	175
16	IRSTRSSDQQQRCCDELNEM	-212	66	-229	150	18,364	3,236	-427	161	-237	171
17	TRSSDQQQRCCDELNEMENT	-231	10	-251	125	20,772	5,265	-418	132	-232	175
18	SDQQQRCCDELNEMENTQRC	-223	31	1,772	434	15,899	4,610	-108	643	703	841
19	QQRCCDELNEMENTQRCMCE	-245	64	503	165	1,439	128	-541	262	-227	200
20	CDELNEMENTQRCMCEALQ	-240	36	281	158	96	132	-508	255	-192	181
21	ELNEMENTQRCMCEALQQIM	-228	19	-17	47	340	209	-149	121	-160	145
22	EMENTQRCMCEALQQIMENQ	-229	28	-224	107	-150	94	-466	303	-219	163
23	NTQRCMCEALQQIMENQCDR	-235	37	750	26	8,339	881	141	260	-173	231
24	RCMCEALQQIMENQCDRLQD	-255	31	238	51	7,643	653	-466	296	-150	164
25	CEALQQIMENQCDRLQDRQM	-164	48	458	18	9,784	2,230	-415	263	382	330
26	LQQIMENQCDRLQDRQMVQQ	-202	14	-2	98	1,695	862	-551	303	54	349
27	IMENQCDRLQDRQMVQQFKR	-184	83	408	390	5,504	3,110	-373	215	268	209
28	NQCDRLQDRQMVQQFKRELM	-245	45	26	131	10,213	5,142	-464	130	-38	324
29	DRLQDRQMVQQFKRELMNLP	9,062	1,228	8,349	1,370	21,265	1,945	-436	142	215	111
30	QDRQMVQQFKRELMNLPQQC	1,225	216	1,892	766	3,607	1,234	-144	144	9	25
31	QMVQQFKRELMNLPQQCNFR	1,102	238	1,940	1,055	2,403	928	974	1,059	-56	27
32	QQFKRELMNLPQQCNFRAPQ	762	164	973	332	1,423	849	-212	419	1,031	414
33	KRELMNLPQQCNFRAPQRCD	2,650	1,544	2,341	1,282	5,335	1,242	24	92	482	328
34	LMNLPQQCNFRAPQRCDLDV	-77	93	-40	143	10,118	1,179	-446	318	-204	122
35	LPQQCNFRAPQRCDLDVSGG	-223	4	-174	142	5,957	4	-527	255	-200	135
36	QCNFRAPQRCDLDVSGGRC	-191	14	-210	156	5,763	6,101	-569	281	-121	250
nAra h 6	Native Ara h 6	12,032	3,711	14,008	3,709	16,611	1,961	5,703	3,463	6,373	1,997

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

Table S3E. Ara h 6: Intensities for sera #21-25

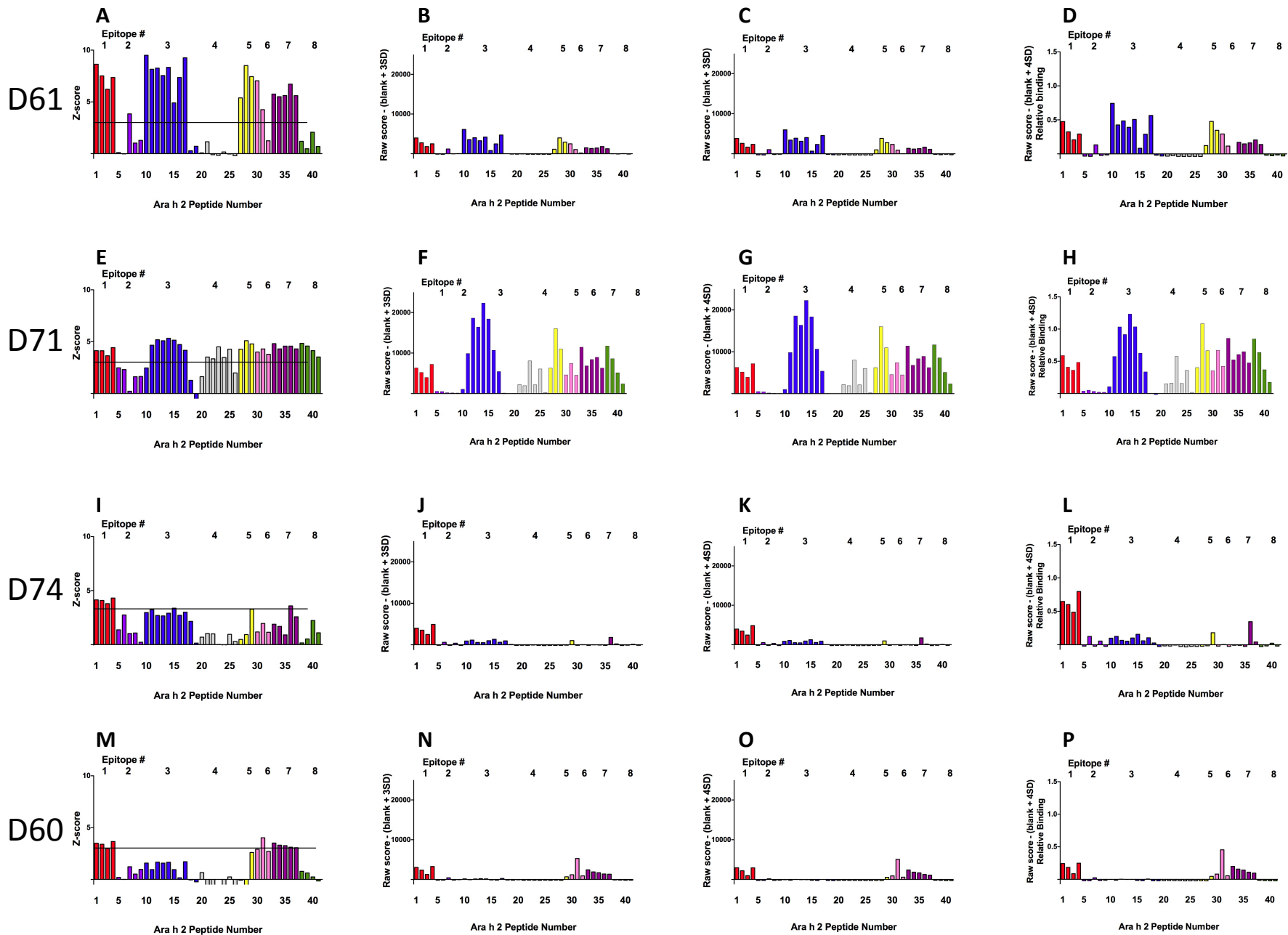
Peptide	Sera:	Intensity									
		D77		D78		D80		D81		D82	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	MRRERG RQGDSSS CERQVDR	331	33	850	100	3,268	2,540	-297	267	-306	182
2	ERG RQGDSSS CERQVDRVNL	-108	195	-131	46	-282	108	-286	230	-339	152
3	RQGDSSS CERQVDRVNLKPC	-178	111	-114	8	218	366	-236	257	-250	156
4	DSSSCERQVDRVNLKPCEQH	-234	79	-75	47	31	89	-308	198	-282	173
5	SCERQVDRVNLKPCEQHIMQ	432	547	-111	13	-122	98	-226	211	-61	43
6	RQVDRVNL KPCEQHIMQRI M	-105	85	81	82	439	51	290	786	-343	160
7	DRVNL KPCEQHIMQRI MGEQ	-35	1	-114	3	-159	148	-129	372	-217	170
8	NL KPCEQHIMQRI MGEQEY	-192	70	-139	20	-28	83	-257	286	-250	255
9	PCEQHIMQRI MGEQEYDSY	-264	44	-106	38	-270	112	-267	272	-238	199
10	QHIMQRI MGEQEYDSYDIR	-287	23	-162	13	2,711	1,433	-162	361	5	182
11	IMGEQEY QYDSYDIR STR	-182	90	-158	10	6,348	1,951	-198	338	-319	144
12	IMGEQEY QYDSYDIR SSD	-272	21	-108	4	7,229	1,817	-259	301	-313	164
13	EQEQY QYDSYDIR SSDQQ	-302	17	-173	31	3,753	1,122	-261	291	-75	13
14	QYDSYDIR STRSSDQQRC	-288	21	-96	6	4,670	731	-144	188	-255	207
15	SYDIRSTRSSDQQRCDEL	-308	16	-190	25	1,847	239	-281	214	-293	206
16	IRSTRSSDQQRCDELNEM	-268	14	-124	27	-202	80	-164	175	-247	231
17	TRSSDQQRCDELNEMENT	-269	20	-156	45	-219	112	-259	187	-250	241
18	SDQQRC DELNEMENTQRC	-190	115	-136	33	-214	77	-138	85	-298	188
19	QQRC DELNEMENTQRC MCE	-218	45	-182	25	-284	152	-289	233	-305	172
20	CDELNEMENTQRC MCEALQ	-251	0	-118	28	-271	148	-152	66	-272	196
21	ELNEMENTQRCMCEALQQIM	-222	21	-154	21	-255	93	-160	278	-302	175
22	EMENTQRCMCEALQQIMENQ	-245	41	-169	36	-195	23	-309	249	-313	176
23	NTQRCM CEALQQIMENQCD R	-22	93	-175	23	-280	122	-324	185	-334	145
24	RCM CEALQQIMENQCD RQLQD	-23	91	-177	6	-244	81	-328	206	-353	153
25	CEALQQIMENQCD RQLQDRQM	130	30	-88	2	-175	51	-219	154	-330	166
26	LQQIMENQCDRLQDRQMVQQ	285	482	-131	20	-139	170	-285	250	-324	139
27	IMENQCDRLQDRQMVQQFKR	517	702	130	293	-209	177	159	127	-304	151
28	NQCDRLQDRQMVQQFKRELM	-253	18	-74	50	-139	157	-85	318	-172	327
29	DRLQDRQMVQQFKRELMNLP	-211	14	-128	3	701	400	1,478	776	578	280
30	QDRQMVQQF KRELMNLPQQC	-296	22	-116	8	-173	113	248	374	-267	191
31	QMVQQF KRELMNLPQQ CNFR	-225	30	-126	33	-124	233	324	721	-294	174
32	QQF KRELMNLPQQ CNFRAPQ	1,853	1,028	-68	8	-89	34	-136	303	-136	326
33	KRELMNLPQQ CNFRAPQRCD	513	465	-98	1	-17	105	267	157	-234	184
34	LMNLPQQ CNFRAPQRCDLDV	-270	25	-170	42	-100	39	-334	184	-328	179
35	LPQQ CNFRAPQRCDLDV SGG	-305	46	-168	8	-245	92	-309	275	-285	148
36	QCNFRAPQRCDLDV SGGRC	-287	49	-115	16	-268	107	-272	165	-308	127
nAra h 6	Native Ara h 6	7,342	3,130	13,793	1,715	19,496	2,670	13,806	8,589	10,856	246

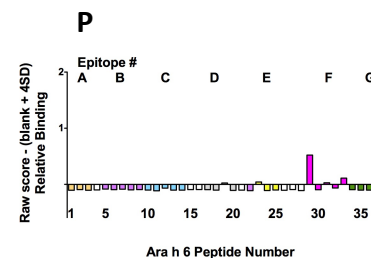
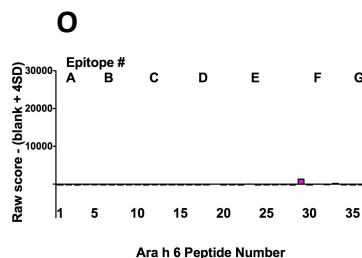
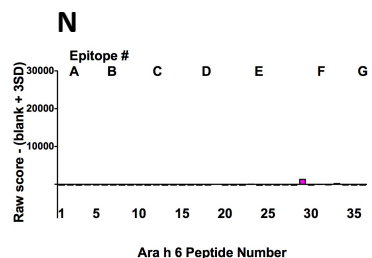
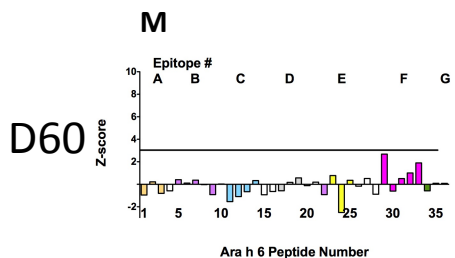
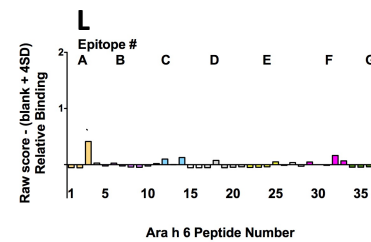
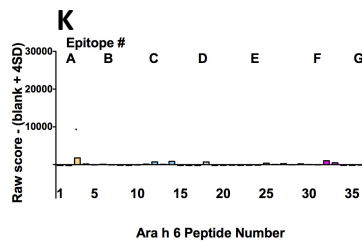
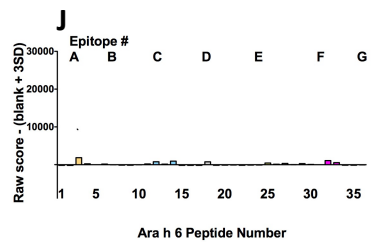
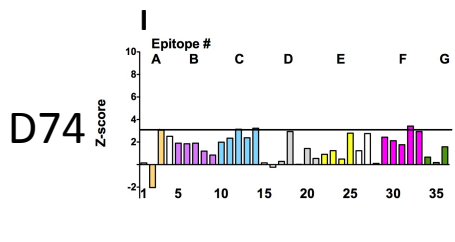
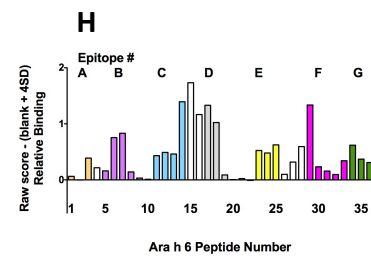
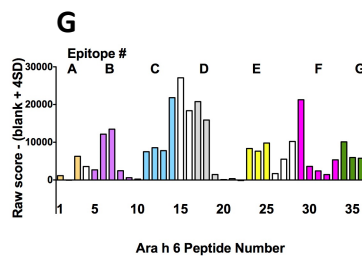
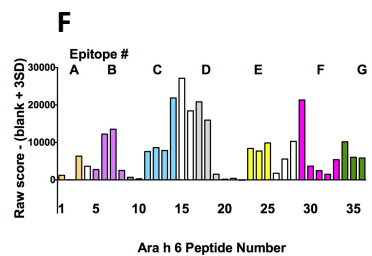
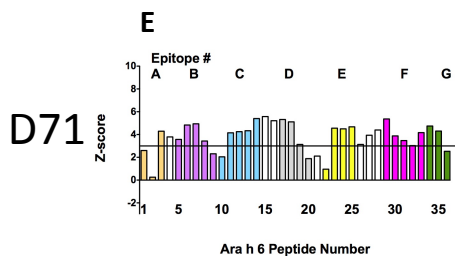
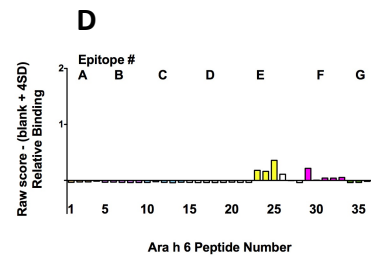
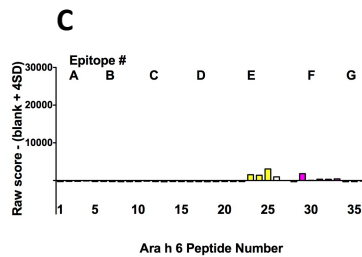
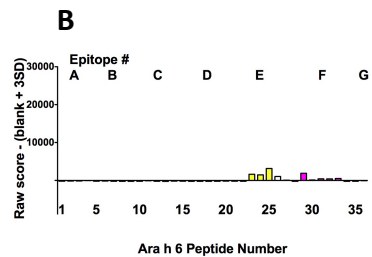
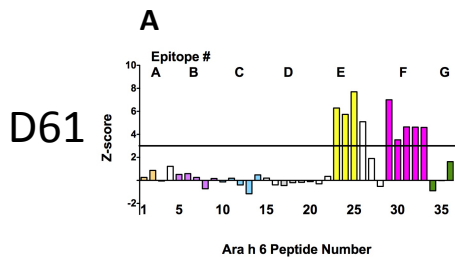
Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)

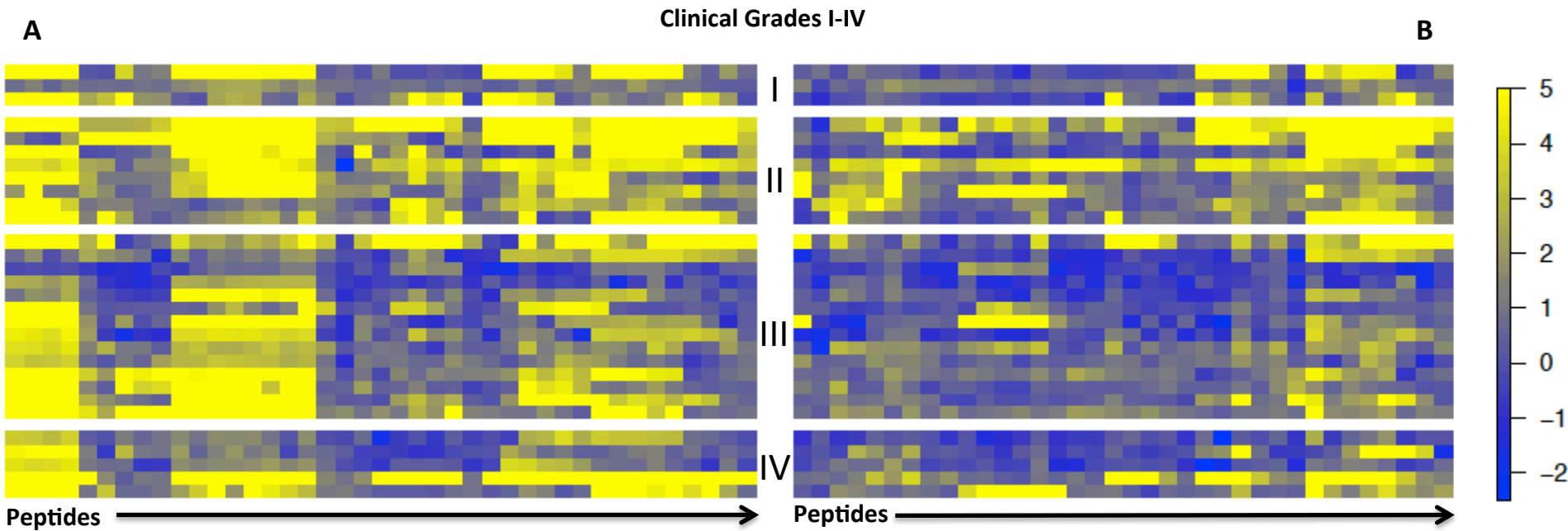
Table S3F. Ara h 6: Intensities for sera #26-30

Peptide	Sera:	Intensity									
		D98		D103		D105		D107		D114	
	Sequence	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	MRRERG RQGDSSS CERQVDR	-494	312	-207	28	-556	17	-204	62	-313	95
2	ERG RQGDSSS CERQVDRVNL	-503	266	-301	98	101	725	-343	108	-485	248
3	RQGDSSS CERQVDRVNLKPC	-356	257	776	379	265	330	-290	122	-412	158
4	DSSSCERQVDRVNLKPCEQH	-414	285	-303	66	133	106	-306	120	-430	185
5	SCERQVDRVNLKPCEQHIMQ	-468	317	-242	189	362	794	-316	81	-378	200
6	RQVDRVNL KPCEQHIMQRI M	-483	332	620	865	883	23	-279	94	-276	57
7	DRVNL KPCEQHIMQRI MGEQ	-190	76	-176	250	-330	115	-366	176	-453	226
8	NL KPCEQHIMQRI MGEQEY	-196	6	-371	120	-463	80	-412	194	-474	241
9	PCEQHIMQRI MGEQEY DSY	-462	254	-269	146	-522	85	-480	176	-471	226
10	QHIMQRI MGEQEY DSYDIR	23	57	-276	156	-493	52	-490	175	-406	287
11	MQRIMGEQEY DSYDIR STR	2,124	83	-335	81	-500	95	-464	200	-471	235
12	IMGEQEY DSYDIR STRSSD	5,717	160	-389	74	-491	45	-466	211	-463	222
13	EQEQ DSYDIR STRSSDQQQ	1,072	109	-365	21	-365	197	-461	215	-473	220
14	QDSYDIR STRSSDQQQRCC	1,278	158	-280	38	-160	106	-436	187	-450	231
15	SYDIRSTRSSDQQQRCCDEL	669	257	-327	31	-533	118	-464	196	-463	209
16	IRSTRSSDQQQRCCDELNEM	-415	296	-295	129	-548	80	-402	170	-460	238
17	TRSSDQQQRCCDELNEMENT	-425	299	-340	100	-422	139	-446	197	-481	231
18	SDQQQRCCDELNEMENTQRC	-273	153	-324	77	-359	86	-373	252	226	441
19	QQRCCDELNEMENTQRCMCE	-474	286	-337	48	-520	37	-456	209	-401	274
20	CCDELNEMENTQRCMCEALQ	-482	249	-296	45	-507	31	-455	199	-419	259
21	ELNEMENTQRCMCEALQQIM	-388	210	-187	130	-511	30	-402	153	-468	251
22	EMENTQRCMCEALQQIMENQ	-428	244	-314	25	-415	143	-363	71	-476	248
23	NTQRCM CEALQQIMENQCD R	-442	264	-322	85	-429	234	-109	63	121	421
24	RCM CEALQQIMENQCD RLQD	-448	307	-309	111	-72	239	-296	88	-265	260
25	CEALQQIMENQCD RLQDRQM	-281	345	-41	296	-95	115	2,223	193	-204	253
26	LQQIMENQCDRLQDRQMVQQ	-494	305	-145	232	-341	124	-331	72	-350	212
27	IMENQCDRLQDRQMVQQFKR	-457	321	155	286	-324	18	-374	178	-408	170
28	NQCDRLQDRQMVQQFKRELM	288	482	399	78	-301	161	2,737	735	-468	226
29	DRLQDRQMVQQFKRELMNLP	1,098	213	617	808	-377	183	104	43	-240	299
30	QDRQMVQQF KRELMNLPQQ C	-111	90	-32	398	-384	223	-339	116	-441	233
31	QMVQQF KRELMNLPQQ CNFR	-278	136	1,073	1,441	-129	467	-236	1	-393	274
32	QQF KRELMNLPQQ CNFRAPQ	-202	201	526	964	263	187	-224	91	-378	248
33	KRELMNLPQQ CNFRAPQRCD	-70	240	272	757	-28	71	843	145	-238	243
34	LMNLPQQ CNFRAPQRCD LDV	-277	266	-275	90	-316	91	-34	280	-88	323
35	LPQQ CNFRAPQRCD LDVSGG	-342	198	-342	47	-455	138	-301	131	-344	240
36	QCNFRAPQRCD LDVSGGRC	-273	231	-336	8	-496	72	-466	223	-418	222
nAra h 6	Native Ara h 6	12,662	515	6,190	3,434	2,443	95	8,386	1,804	4,488	163

Intensity = (mean of the raw values of two assays) minus (the mean of the empty spots + 4 SD of the empty spots)







A

Color Key



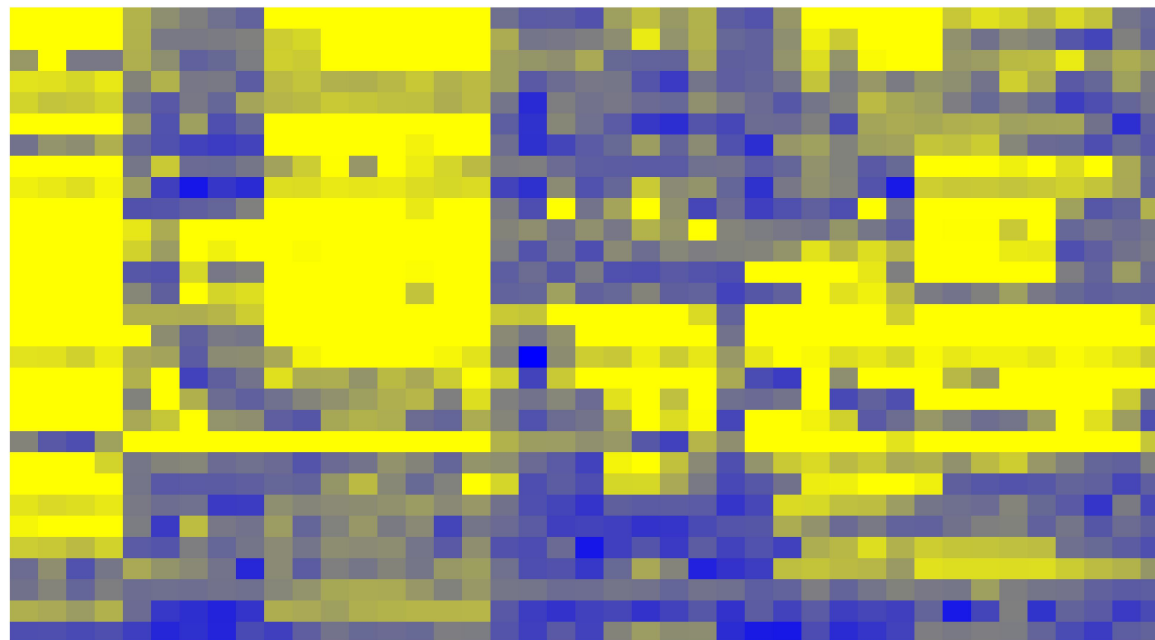
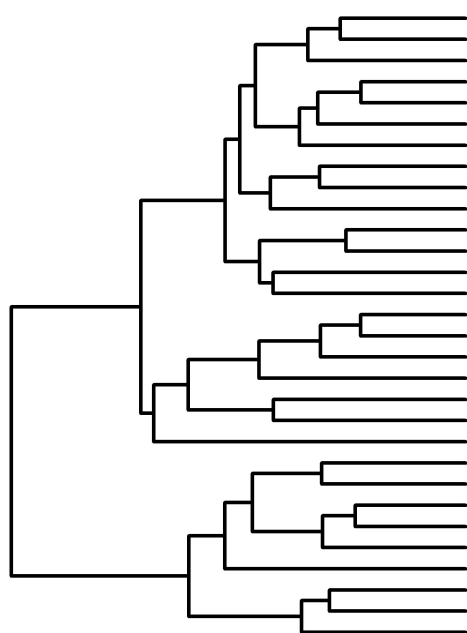
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2

4

Value



Peptides

Subjects

D107

D78

D80

D74

D77

D66

D63

D98

D67

D70

D82

D81

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D103

D48

D70B

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D44

D19

D114

D64

D105

D65

D69

D68

D60

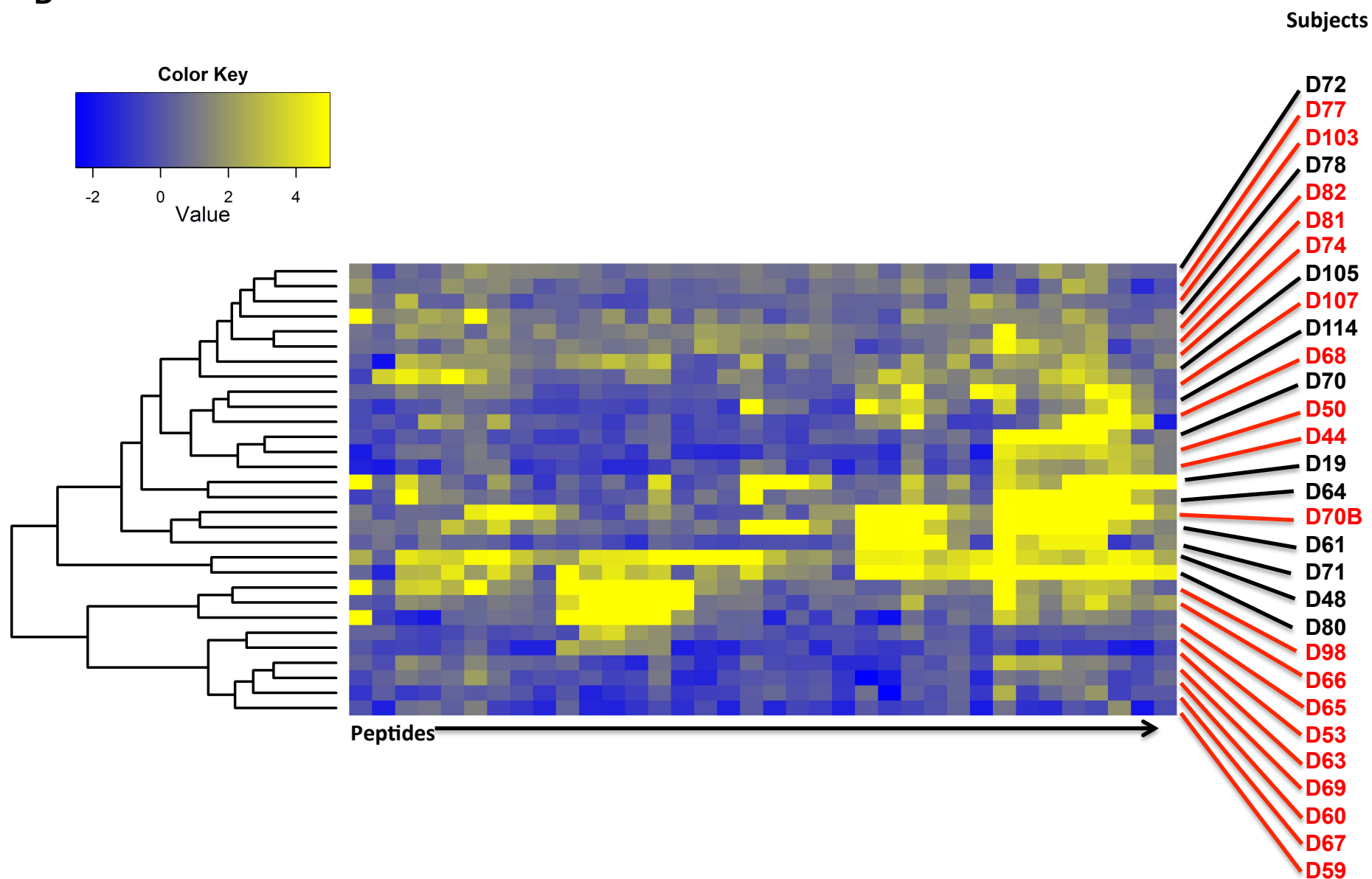
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D72

D59

D53

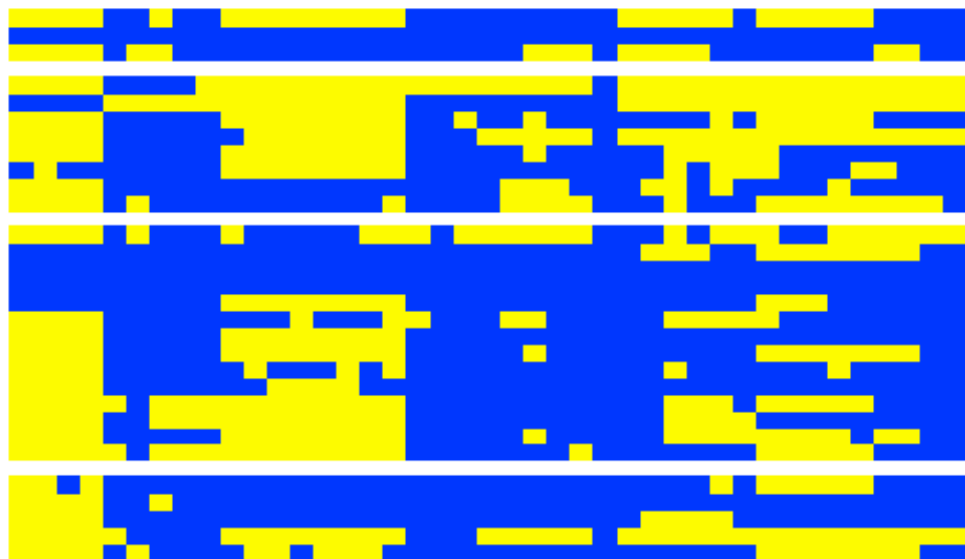
B



A

Clinical Grades I-IV

B



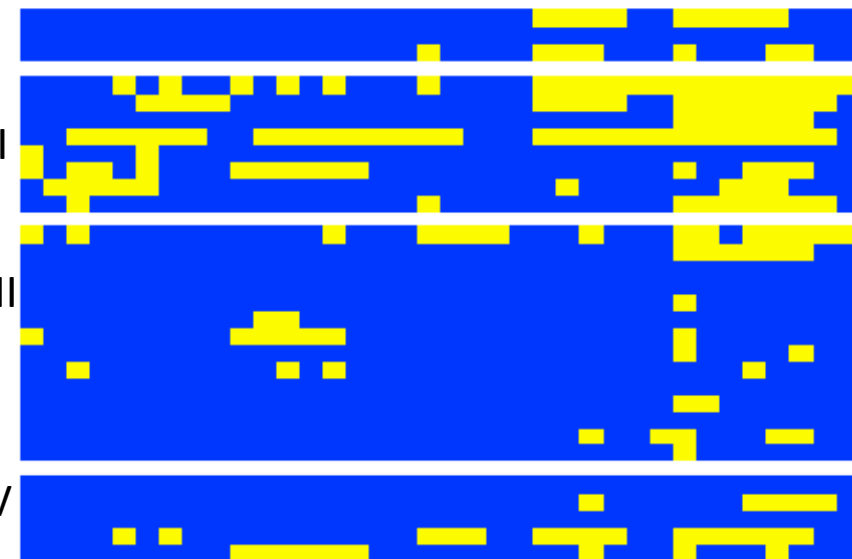
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II

III

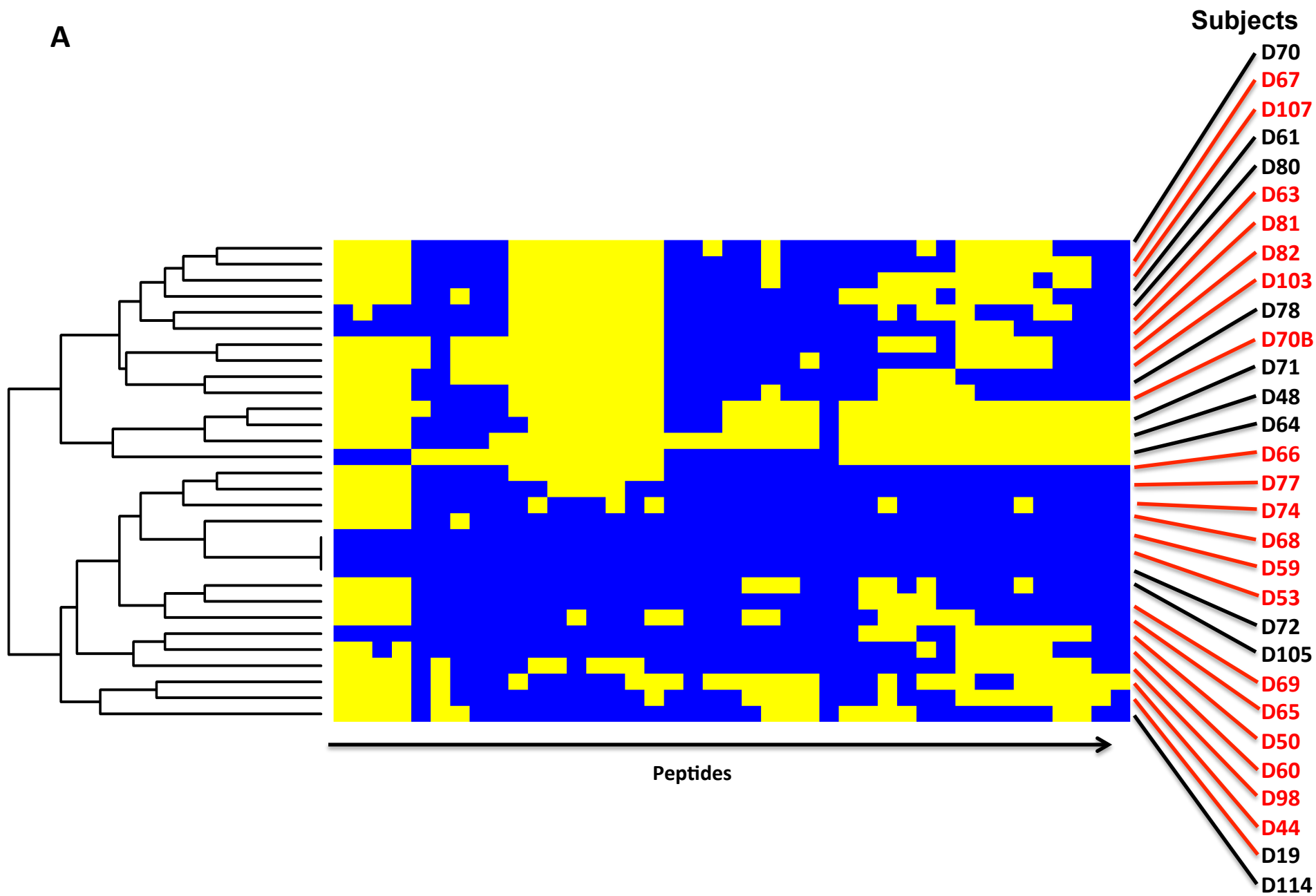
IV

Peptides



Peptides

A



B

Subjects

D64

D61

D70B

D48

D44

D71

D70

D50

D19

D107

D68

D114

D82

D63

D59

D53

D60

D69

D72

D77

D103

D67

D81

D65

D78

D74

D105

D98

D66

D80



Peptides