

IgE and IgG₄ epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2

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Background: Detailed assessment of antibody responses to allergens reveals clinically relevant information about both host response and antigen structure. Microarray technology offers advantages of scale and parallel design over previous methods of epitope mapping.

Objective: We designed a redundant peptide microarray for IgE and IgG₄ epitope mapping of the previously characterized peanut allergen, Ara h 2.

Methods: Six complete sets of overlapping peptides were commercially synthesized and site-specifically bound to epoxy-derivatized glass slides in triplicate. Peptides were 10, 15, or 20 amino acids in length with an offset of either 2 or 3 amino acids. A total of 10 control and 45 peanut-allergic sera were assayed. Specific IgE and IgG₄ were detected by using fluorochrome-labeled monoclonal secondary antibodies.

Results: By using 15-mer and 20-mer peptides, we could define 11 antigenic regions, whereas only 5 were identifiable using 10-mers. Controls and patients produced IgG₄ recognizing a comparable number of Ara h 2 peptides, although the dominant epitopes were distinct. As expected, patient IgE bound a larger number of Ara h 2 peptides (9.4% vs 0.9%). IgE and IgG₄ epitopes recognized by patients were largely the same, and there was a positive association between IgE and IgG₄ signal, suggesting coordinate regulation. Cluster analysis of peptide binding patterns confirmed the specificity of antibody-peptide interactions and was used to define 9 core epitopes ranging from 6 to 16 residues in length—7 of which (78%) agreed with previous mapping.

Conclusion: Epitope mapping by microarray peptide immunoassay and cluster analysis reveals interpatient heterogeneity and a more detailed map. (*J Allergy Clin Immunol* 2005;116:893-9.)

Key words: Peptide/protein microarray, Ara h 2, epitope mapping, antibody diversity, IgE, IgG₄, peanut allergy

Antigen-specific B-cell responses are accessible to study with minimal invasiveness because of the presence of their specific antigen receptors in relatively abundant quantities in serum and their capacity to recognize cognate antigen directly.¹ The investigation of antibody responses may reveal information about both host response and antigen structure.

The measurement of a polyclonal B-cell response to a complex antigen is the sum of several variables, including the concentration of each specific antibody clone, the affinity of each clone, and the clonal diversity that together determine the fraction of target (antigen) that is bound.²⁻⁵

Diversification, along with affinity maturation and class switch recombination, is the result of immune progression—a process that is specifically regulated⁶⁻⁸ and may provide insight into the host immune response. For example, clinical correlations have been made among antibody diversity and clinical autoimmunity,⁹ anti-infective immunity,¹⁰ and clinical allergy.¹¹⁻¹³ We have previously shown a correlation between specific IgE diversity and clinical allergy severity as well as *in vitro* effector function.¹⁴

Antigen arrays can be used to measure clonal diversity.¹⁵ As the antigenic complexity of each arrayed element decreases, fewer epitopes are represented, and therefore, fewer distinct clonal responses are measured—approaching a theoretical limit of 1 array element to 1 antibody clone. This can be accomplished by using short synthetic peptides derived from the primary sequence of the corresponding antigen of interest.

Synthetic peptide arrays may also be used to elucidate antigenic structure. Investigators¹⁶ including Geysen et al^{17,18} pioneered the use of peptide arrays to measure the polyclonal response in outbred animals to study epitope structure. Many investigators have used small overlapping arrays of peptides to define continuous B-cell epitopes of numerous antigens, including many allergens.^{13,19-27}

Because of its ability to assay thousands of targets in parallel by using small volumes of sample, the microarray immunoassay is ideally suited for the determination of individual polyclonal epitope recognition patterns.²⁸ Furthermore, signal intensity for microarray-based immunoassays is more dependent on affinity and tolerant of low concentration,³⁻⁵ making it an ideal platform for the

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Supported by the Mount Sinai Medical Center General Clinical Research Center National Institutes of Health Division of Receipt and Referral M01-RR-00071 and the Food Allergy Initiative. Dr Shreffler is supported by the National Institutes of Health Loan Repayment Program; Dr Shreffler and Dr. Sampson are supported by National Institutes of Health P01-AI44236-01.

Disclosure of potential conflict of interest: H. Sampson is a consultant for Seer Pharmaceuticals, Inc, and is employed by Mount Sinai School of Medicine. D. Lencer, L. Bardina, and W. Shreffler are employed by Mount Sinai School of Medicine.

Received for publication April 29, 2005; revised June 9, 2005; accepted for publication June 10, 2005.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2005.06.033

Abbreviations used

dfu: Digital fluorescence units
 HAS: Human serum albumin
 PBS-T: PBS with 0.5% Tween 20

detection of epitope-specific IgE and IgG₄—immunoglobulins present at low concentrations in serum.

We sought to adapt protein microarray technology for the measurement of antibody clonal diversity and characterization of antigenic structure in the context of a T_H2-skewed immune response to a major dietary antigen, Ara h 2, from peanut (*Arachis hypogaea*). Ara h 2 is an abundant 17/19-kD glycoprotein member of the lipid transfer protein family of seed storage proteins that is widely recognized by specific IgE from individuals allergic to peanut.^{14,29-32}

We describe here the novel use of the microarray immunoassay with hierarchical cluster analysis to define IgE and IgG₄ epitopes of a major dietary allergen. We also compare the sensitivity of peptides of varying lengths for the detection of specific antibody on this substrate.

METHODS

Peptides, reagents, and array production

Six sets of peptides 10, 15, and 20 amino acids in length with an offset of 2 or 3, covering the sequence of the major peanut allergen, Ara h 2 (GeneBank accession, AAK96887), were commercially synthesized (Mimotopes, Pty Ltd, Raleigh, NC).

Stock solutions of peptides were dissolved in 40% acetonitrile, 20% dimethyl sulfoxide, to 1.6 mol/L. Working dilutions were then made in Protein Printing Buffer (Telechem, Sunnyvale, Calif) to a final concentration of 100 μmol/L and stored in 384-well polypropylene plates (Matrix Technologies, Hudson, NH). Triplicate spots were printed on epoxy-derivatized slides (SuperEpoxy Substrate, Telechem, Sunnyvale, Calif) 350 nm apart by using a GMS Arrayer (Affymetrix, Inc, Santa Clara, Calif). AlexaFluor-labeled protein was used to print positional reference controls. Negative control features (buffer alone and nonbinding peptides) and positive control (recombinant Ara h 2³³) were also printed. Arrays were stored in a desiccator until use. Identical 2304-feature arrays were used for all experiments.

Anti-IgE and Anti-IgG₄ mAbs (both Pharmingen, San Jose, Calif) covalently conjugated to fluorescent markers (AlexaFluor 647 and 546, respectively) were prepared by reaction with activated *N*-hydroxysuccinimidyl-ester fluorochromes according to the manufacturer's protocol (Molecular Probes, Eugene, Ore). Final molar ratios of fluorochrome to immunoglobulin were between 5 and 9.

Forty-five patient sera with documented clinical reactivity and high peanut specific IgE values (median > 100 kU/L; Immuno CAP System FEIA; Pharmacia, Uppsala, Sweden) were recruited from the Mount Sinai Allergy Clinic. Ten nonatopic control sera were taken from healthy volunteers with no history of food adverse reactions.

Immunolabeling

An area around the printed grids was encircled with a hydrophobic PAP Pen (DAKO, Glostrup, Denmark) and blocked for 1 hour with 200 μL PBS with 0.5% Tween 20 (PBS-T) with 1% human serum

albumin (HSA) at room temperature. Diluted patient serum (1:5 in PBS-T/HSA) was applied to the array and was incubated overnight on a rotator at ambient temperature. Titration (see Fig E1 in the Online Repository in the online version of this article at www.jacionline.org) and kinetics experiments (not shown) were previously performed to determine optimum conditions. Slides were then washed with PBS-T and incubated for 150 minutes with 200 μL anti-IgE and anti-IgG₄ cocktail (1:16,000 PBS-T/HSA) at room temperature in the dark. All incubations were performed in a humidity chamber (Binding Site, San Diego, Calif). Subsequently, the slides were washed 3 times for 3 minutes in PBS-T in a washing station (Telechem, Sunnyvale, Calif), followed by 3 times rinsing in deuterium, and spun dry.

Analysis

The feature and background pixel intensities were determined with ScanAlyze software (Michael Eisen; <http://rana.lbl.gov/EisenSoftware.htm>), and the net signal was exported and expressed as digital fluorescence units (dfu). A Microsoft Excel Visual Basic Application (Microsoft, Renton, Wash) created by our laboratory was used to relate the grid data to the corresponding peptide identity and to calculate the means and SDs of the triplicates. Array elements with coefficient of variance > 30% were excluded from analysis. The mean plus 2 SD log-transformed of the buffer alone negative controls was used as an intraslide cutoff to establish background. An interslide cutoff was calculated as the mean plus 2 SD intensity of the corresponding signal from the nonatopic control sera. For calculation of percent of patients with positive IgE binding, both intraslide and interslide cutoffs had to be exceeded. For hierarchical cluster analysis, the IgE/IgG₄ ratio of log-transformed data from all 55 patients and controls on all peptide elements was used. Clusters were determined by using Cluster software (<http://rana.lbl.gov/EisenSoftware.htm>) using the complete linkage algorithm and centered measurements for both arrays and features. Results are displayed by using Java TreeView 1.0.8 (<http://rana.lbl.gov/EisenSoftware.htm>). Additional analysis and graphical display were performed by using Prism (GraphPad Software, San Diego, Calif).

RESULTS

Commercially synthesized peptides of different lengths (10, 15, or 20 amino acids) and different degrees of overlap (2 or 3 offset), corresponding to the sequence of Ara h 2, were robotically arrayed in triplicate and site-specifically attached to derivatized glass slides; we assayed these peptides in parallel with sera from 45 individual patients allergic to peanut and 10 nonallergic controls.

Fig 1 displays scatter plots of IgE versus IgG₄ signal for negative control array elements probed with patient sera (A), Ara h 2 peptide array elements probed with nonallergic control sera (B), or Ara h 2 peptide array elements probed with patient sera (C). As can be seen from Fig 1, A, a low level of background signal because of autofluorescence and nonspecific primary or secondary antibody binding exists (<0.5% for each channel). The central tendency of signal for negative control elements (for both controls and patients), normal control sera on peptide elements, or patients on peptide elements is the same—that is, a minority of antibody peptide interactions results in a positive signal. From controls not allergic to peanut,

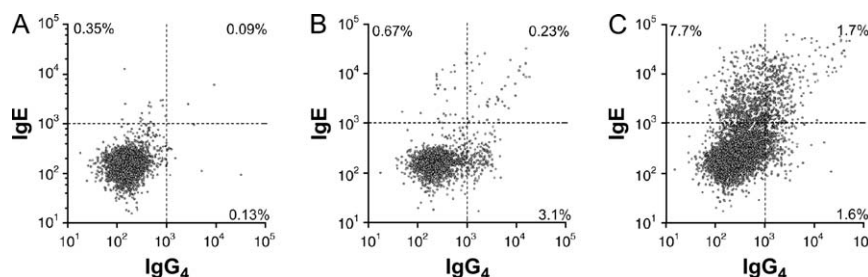


FIG 1. IgE and IgG₄ fluorescence signal on microarray elements. **A**, Patient sera on negative control array elements. **B**, Nonallergic control samples probed with Ara h 2 peptide array elements. **C**, Allergic patient samples probed with Ara h 2 peptide array elements.

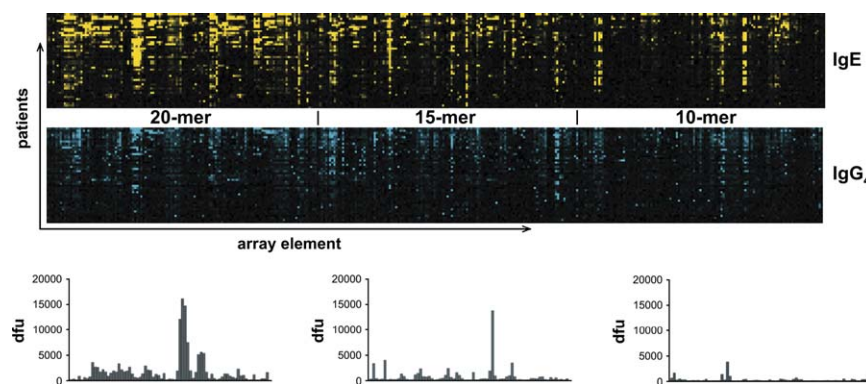


FIG 2. Comparison of Ara h 2-derived peptides of 10, 15, or 20 residues for the detection of specific IgE and IgG₄. *Top*, IgE (yellow) and IgG₄ (blue) reactivity to overlapping Ara h 2 peptides of indicated length. Each row indicates signal from 1 patient ($n = 45$). *Bottom*, Average signal intensity (in dfu) plotted from N-terminal to C-terminal position for 20-mer (left), 15-mer (center), or 10-mer (right) Ara h 2 peptides.

IgG₄ fluorescence signals above the arbitrary cutoff of 10^3 dfu ($>$ mean plus 2 SD $\log_{10}$ -transformed data from negative control elements) were present in approximately 3.3% (131/3940) of all potential Ara h 2 peptide–antibody interactions (Fig 1, B), suggesting that nonallergic individuals do produce IgG₄ antibody specific to sequential allergen epitopes. IgE signal was low ($<1\%$).

In comparison, assay with individuals allergic to peanut also produced positive IgG₄ signal to 3.3% (although with higher mean fluorescence) but IgE signal to 9.4% of Ara h 2 peptide–antibody interactions ($n = 17,732$) above the same threshold. About half of all IgG₄-positive events were also IgE-positive, whereas the majority of IgE-positive events were IgG₄-negative (7.7%, IgE only; 1.7%, both IgE and IgG₄). There was a highly significant degree of association between IgG₄ and IgE signal from patient sera on peptide elements. To assess this association better, we restricted the analysis to include only peptide elements giving a positive IgE or IgG₄ signal from at least 5 of 45 patients ($n = 9405$). Of these, 423 were positive for both IgE and IgG₄, versus 169 double-positive events that would be expected on the basis of the frequency of IgE and IgG₄ alone ($\chi^2 = 535$; $P = 2.16 \times 10^{-118}$).

If the measured reactivity is sequence-specific, then we should expect to see a non-random distribution of reactivity from overlapping peptides although they were printed

at physically disparate addresses on the array. Fig 2, A, displays antibody signal as color intensity (yellow for IgE, blue for IgG₄) with peptides ordered by sequence position in columns (all 6 sets of Ara h 2 peptides) and patients in rows ($n = 45$). As expected, positive signals are present on distinct sets of overlapping peptides, consistent with the recognition of a specific sequence. Fig 2, B, displays the mean dfu for all 45 patients at each peptide element of the 3 sets of 2 offset peptides. As expected, positive signals occur in peaks of reactivity to overlapping peptides.

Comparing detectable antibody signal to peptides of 10, 15, or 20 amino acids, it is clear that signal intensity for both IgE and IgG₄ is increased as a function of peptide length. This suggests higher affinity interactions or the presence of multiple epitopes on longer peptides. Analysis of the 2-offset set of peptides for specific IgE binding (ie, above intraslide background and above nonallergic control binding) reveals 5 distinct maxima involving 6 of 81 (7.4%) of 10-mers that are specifically recognized by more than 20% of patients. In contrast, 11 peaks involving 20 of 76 (26.3%) and 11 involving 31 of 73 (42.4%) are identified by using 15-mers and 20-mers, respectively. Three offset peptide sets were equivalent and identified the same antigenic regions (see Fig E2 in the Online Repository in the online version of this article at www.jacionline.org).

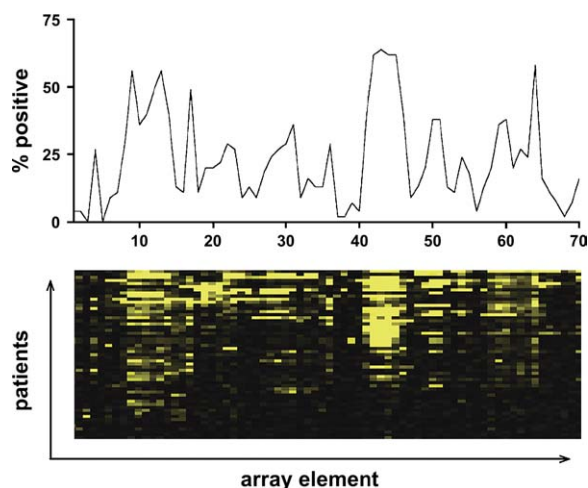


FIG 3. Interpatient heterogeneity of epitope pattern. *Top*, Percent of 45 patients allergic to peanut above intraslide and interslide cutoffs for each 20-mer 2-offset Ara h 2 peptide. *Bottom*, Individual IgE binding to the same peptides. Each row indicates signal from 1 patient ($n = 45$).

Fig 2, A, demonstrates that IgE and IgG₄ epitopes are largely the same on a population level—although IgE binding is stronger in this group of samples and tends to involve broader groups of overlapping peptides (see Fig E3 in the Online Repository in the online version of this article at www.jacionline.org).

As we have previously described, whereas dominant antigenic regions can be identified by using this approach, there is a high degree of interpatient heterogeneity. Individual patient clones may bind a slightly different set of overlapping peptides representing an immunodominant region, whereas some patient IgE clones appear to recognize exceptional regions. As an example, Fig 3 displays the individual heterogeneity in recognition patterns for these 45 patients across the set of 20-residue, 2-offset peptides juxtaposed with the percent positive value plotted against the same peptide array elements. From this comparison, one can see that peak areas of antibody binding are present but that individual patterns of recognition may vary—even through dominant regions.

We hypothesized that if epitope selection is sufficiently constrained, using statistical clustering of binding patterns across this highly redundant set of Ara h 2–derived peptides should allow us to identify sequential epitopes more rigorously as the overlapping sequence of clustered elements. We used hierarchical clustering analysis of IgE/IgG₄ signal ratio for all peptide array elements across all 55 arrays (Fig 4). As expected, nonallergic individuals who had low IgE/IgG₄ signal ratios (blue) to peptide array elements were clustered together, whereas patients were clustered into groups according to their signal pattern on peptide clusters. We selected all array peptide clusters containing 4 or more elements with correlation coefficients of at least 0.5 for further analysis. The 10 nodes meeting these criteria are highlighted in Fig 4. All such clusters identify groups of peptides with overlapping

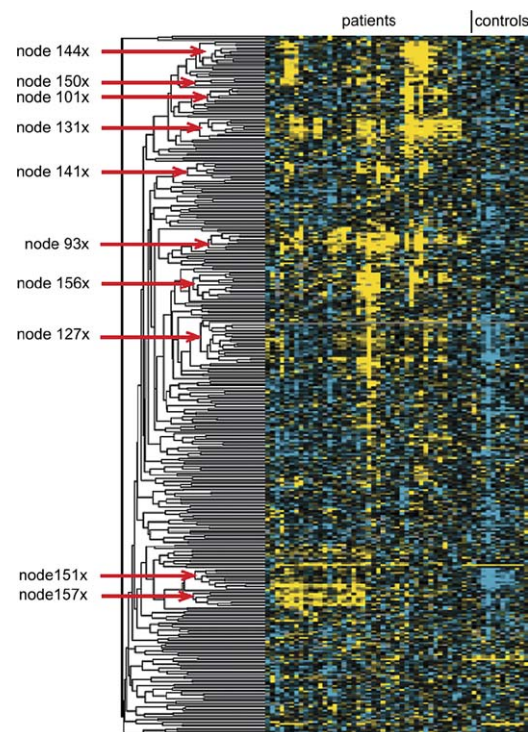


FIG 4. Treeview display of hierarchical cluster analysis of IgE/IgG₄ signal ratio (ratio > 1 in yellow, < 1 in blue). Peptides are in rows and arrays in columns. See Methods for details of cluster analysis. Arrows indicate peptide clusters (nodes) chosen for additional analysis (for additional information, see text and Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

sequence. Five of 10 nodes are composed of a single block of overlapping peptides (nodes 156, 93, 144, 150, 101). An additional 3 contain more than 75% peptides with a single overlapping sequence (nodes 131, 141, 127), whereas the remaining contain at least 5 overlapping peptides comprising 62.5% (node 157) or 45% (node 151) of all peptides. These last 2 nodes are related ($r = 0.4$ between all elements), with 53% (10/19) of the sequences of these 2 nodes containing a 6-amino acid overlap, and are considered as a single node in subsequent analysis (node 212). The sequences of all selected peptides are shown in Table E1 (see the Online Repository in the online version of this article at www.jacionline.org). From alignment of the overlapping sequences of each node, 9 dominant epitopes are identified (see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). In some cases, additional sequences within a cluster contained distinct overlapping sequences (eg, node 212x; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org).

Minimal core overlapping sequences were 6 to 16 (mean, 10) amino acids in length, in good agreement with the length of previously defined B-cell epitopes. To assess the significance of clustering, we binned data at each array element as either positive or negative and performed χ^2

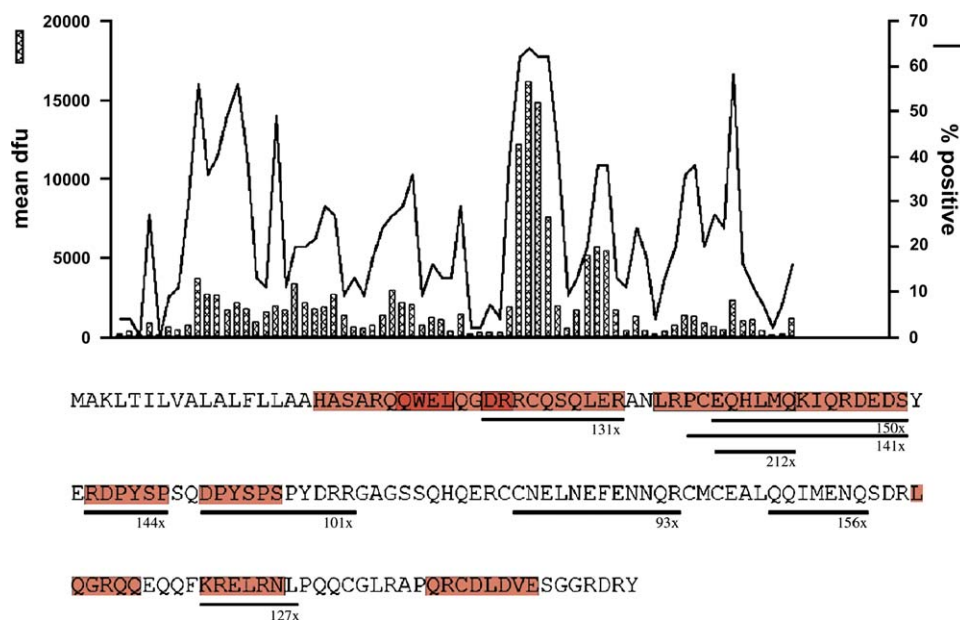


FIG 5. Top, Comparison of average signal intensity versus percent above intraslide and interslide cutoff. Bottom, Comparison of Ara h 2 epitopes identified by cluster analysis (black bars) with previously defined epitopes (highlighted in red²⁶).

analysis for frequency of agreement at clustered elements versus expected agreement of normally distributed data with the same mean number of agreement or versus the data from the same array elements that had been randomized. All clustered nodes were highly statistically significant (eg, node 156, $P = 2.08 \times 10^{-37}$ or $P = 1.45 \times 10^{-10}$ for normally distributed or randomized data, respectively).

Sequential IgE epitopes of Ara h 2 have been previously determined by immunoassay with pooled patient serum against 19 overlapping peptides (15 residues in length, overlapping by 8) synthesized on cellulose membranes.^{26,34} Of the 9 epitopes defined by cluster analysis, 7 (78%) overlap substantially with antigenic regions defined previously (Fig 5).

DISCUSSION

Peptide/protein microarrays have many potential applications in biomedicine and diagnostics. We have been interested in the specificity and diversity of the IgE response to allergens because these features may have diagnostic and prognostic potential for clinical allergy. For example, differences in epitope-specific antibody recognition have been demonstrated between patients with transient versus persistent allergy.^{12,13} A correlation also appears to exist between epitope diversity and IgE function as reflected by patient reaction severity and an *in vitro* effector cell assay.¹⁴

The current study was undertaken to define the optimum peptide length and overlap for determination of sequential IgE epitopes of a major peanut allergen,

Ara h 2, by microarray-based immunoassay. We recognized that the redundancy of this set of Ara h 2 peptides provided us a unique opportunity to explore the specificity of antibody-peptide interactions and statistical means of defining dominant epitopes within a sample population.

To determine dominant epitopes, researchers have generally determined minimal overlapping sequences of peptides comprising a single signal peak.^{12,13,20,24,35,36} There are several problems with this approach, however. For example, a set of overlapping peptides may contain members that do not adopt a secondary conformation that is representative of the native epitope even when containing the complete primary sequence. This results in a local signal minimum that may be falsely interpreted as indicative of 2 separate epitopes. Alternatively, a single broad signal peak may be a result of the presence of 2 distinct epitopes recognized within a given population that are not resolved. This second artifact may be avoided by using shorter peptides, but with a concomitant decrease in sensitivity. We hypothesized that both artifacts may be reduced by assaying a large number of overlapping peptides with individual serum samples—an approach that is only practical using a highly parallel assay such as the microarray immunoassay. Using this approach, each individual's polyclonal binding pattern on the peptide array elements is statistically compared by hierarchical clustering to group individuals recognizing a set (node) of peptides that display a shared epitope.

In this study, we have identified 9 putative epitopes of Ara h 2. Seven of these map to areas that have been previously identified as epitopes.²⁶ However, 2 of the strongest epitopes were not previously identified (nodes 93 and 156). In both cases, the epitope length is within the

15-amino acid length of peptides synthesized previously. However, in the previous study, there was only 1 peptide synthesized that contained the entire epitope, as opposed to the multiple peptides displaying the entire epitope on this microarray. In the case of the node 156 epitope, QQIMENQ, the peptide identical to the one synthesized by SPOTS (ALQQIMENQSDRLQG) was also negative on the microarray assay; however, this epitope was identified by other peptides. Furthermore, in many cases, there was a trend toward better reactivity with 20 versus 15 residue peptides. For the node 156 epitope, 73% (8/11) 20-mers with the complete sequence were positive, in contrast with 50% (4/8) of the 15-mers (and 0/3 10-mers). Similarly, for the node 93 epitope, CNELNEFENNQR, only a fraction of peptides containing the epitope was positive. In total, there were 11 peptides displaying the core sequence on the microarray, and 82% were positive (6/8 positive on 20-mer, 3/3 on 15-mer). One apparent advantage of mapping by microarray, therefore, is the ease of using multiple peptides displaying the putative epitope.

The overlapping epitopes of nodes 150, 141, and 212 illustrate the potential power of using clustering of individual reactivity patterns to dissect polyclonal responses. Variations of epitope selection within a population have allowed for the distinction of 3 putative epitopes within an overlapping region (Fig 5).

In addition to clustering of peptides that share a given epitope, patients are clustered by their epitope recognition patterns (Fig 5). We are investigating the clinical significance of these distinct recognition patterns in the context of peanut allergy. Although antigen structure appears to dominate epitope selection,³⁷ we speculate that this may also be influenced by genetic factors such as germline κ , λ , and H-chain loci.

This study is the first to map IgG₄ epitopes of Ara h 2. We have demonstrated that individuals without allergy produce antibodies to sequential epitopes of Ara h 2, although the signal strength and specificity of IgG₄ appear to be distinct (see Fig E4 in the Online Repository in the online version of this article at www.jacionline.org). Furthermore, unlike what has been reported for other allergens,^{19,24,35,38} the sequential IgG₄ epitopes recognized by patients with allergy do not appear to be distinct from IgE epitopes (see Fig E3 in the Online Repository in the online version of this article at www.jacionline.org). This finding is consistent with the hypothesis that regulation of isotype switching is an important checkpoint in the development of clinical allergy versus tolerance and that IgG may act to block IgE access to antigen.

Future studies comparing tolerant versus allergic individuals with respect to specific IgE versus IgG will allow us to test this directly. To this end, it is necessary to demonstrate assay sensitivity in the range of low specific IgE (1–10 ng/mL) that is found in patients with the most challenging diagnostic problems. With the adoption of primary antibody detection by Alexa dye-conjugated mAbs, we can detect peptide specific signal in serum diluted to the equivalent of a total peanut-specific IgE of <2 pg/mL measured by conventional assay (see Fig E1 in

the Online Repository in the online version of this article at www.jacionline.org). Studies comparing these clinical groups are currently underway.

We also anticipate that the microarray immunoassay will be well suited for the surveillance of immune responses during immunotherapy where the development of epitope spreading within the IgE and IgG₄ pool has been observed.³⁹

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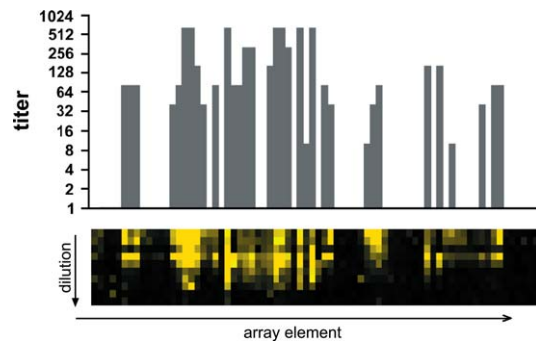


FIG E1. Titration of IgE signal from a serum sample from a single patient with allergy. *Top*, Maximum dilution with positive ($>\text{mean} + 2 \text{ SD}$ of negative control array elements) signal plotted for 20 residue peptides plotted from N-terminal to C-terminal sequence. *Bottom*, Graphical display of signal intensity for these peptide elements on each array (*in rows*) probed with increasingly diluted serum.

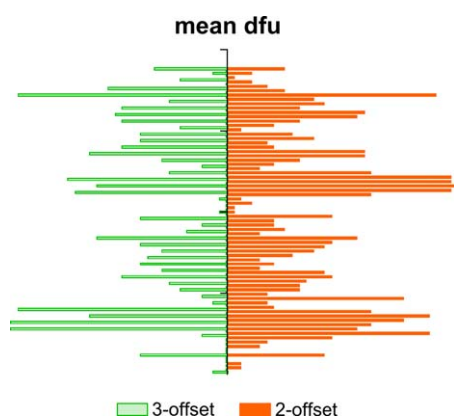


FIG E2. Comparison of IgE signal intensity to 20-mer 2-offset versus 20-mer 3-offset peptides.

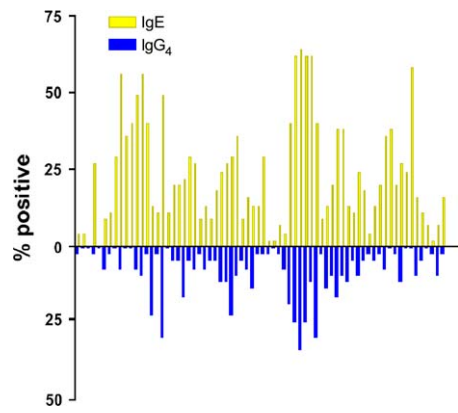


FIG E3. Comparison of IgE (*yellow*) and IgG₄ (*blue*) signal from peanut allergic patients on 20-mer 2-offset peptides.

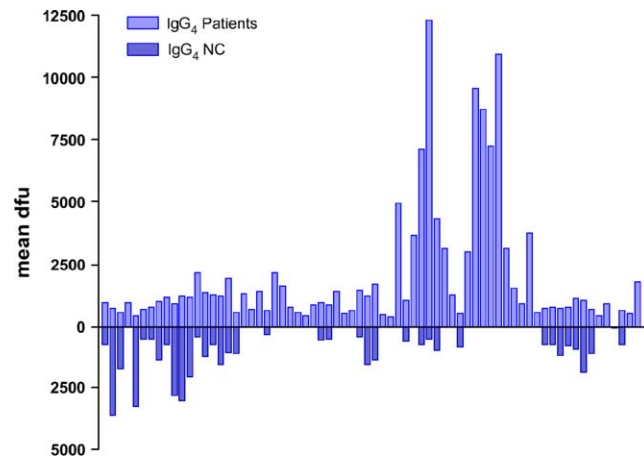


FIG E4. Comparison of mean IgG₄ binding to 20-mer Ara h 2 peptides between sera from a patient allergic to peanut (*light blue*) and a nonallergic control (*dark blue*).

TABLE E1. Overlapping peptides identified by cluster analysis

Peptide	Length	Sequence
node144x	112 15mer	DSYERDPYSPSQDPY
	113 15mer	YERDPYSPSQDPYSP
	114 15mer	RDYSPSQDPYSPSP
	182 20mer	HLMQKIQRDEDSYERDPYSP
	183 20mer	MQKIQRDEDSYERDPYSPSQ
	189 20mer	DSYERDPYSPSQDPYSPSPY
	190 20mer	YERDPYSPSQDPYSPSPYDR
	191 20mer	RDYSPSQDPYSPSPYDRRG
	311 15mer	DSYERDPYSPSQDPY
	312 15mer	ERDPYSPSQDPYSPS
	360 20mer	LMQKIQRDEDSYERDPYSPS
	361 20mer	KIQRDEDSYERDPYSPSQDP
	364 20mer	RDYSPSQDPYSPSQDPYSP
	365 20mer	DSYERDPYSPSQDPYSPSPY
	366 20mer	ERDPYSPSQDPYSPSPYDRR
node150x	104 15mer	EQHLMQKIQRDEDSY
	181 20mer	EQHLMQKIQRDEDSYERDPY
	305 15mer	EQHLMQKIQRDEDSY
	359 20mer	EQHLMQKIQRDEDSYERDPY
node101x	116 15mer	SPSQDPYSPSPYDRR
	117 15mer	SQDPYSPSPYDRRGAG
	118 15mer	DPYSPSPYDRRGAGS
	192 20mer	PYSPSQDPYSPSPYDRRGAG
	194 20mer	SQDPYSPSPYDRRGAGSSQH
	315 15mer	DPYSPSPYDRRGAGS
	367 20mer	PYSPSQDPYSPSPYDRRGAG
	368 20mer	PSQDPYSPSPYDRRGAGSSQ
node131x	94 15mer	QLERANLRPCEQHLM
	168 20mer	SARQQWELQGDRRCQSQLER
	169 20mer	RQQWELQGDRRCQSQLERAN
	170 20mer	QWELQGDRRCQSQLERANLR
	172 20mer	QGDRRCQSQLERANLRPCEQ
	173 20mer	DRRCQSQLERANLRPCEQHL
	298 15mer	WELQGDRRCQSQLER
	351 20mer	RQQWELQGDRRCQSQLERAN
	352 20mer	WELQGDRRCQSQLERANLRP
	266 10mer	AGSSQHQCRC
	268 10mer	QERCCNELNE
node141x	178 20mer	ANLRPCEQHLMQKIQRDEDS
	179 20mer	LRPCEQHLMQKIQRDEDSYE
	180 20mer	PCEQHLMQKIQRDEDSYERD
	357 20mer	ANLRPCEQHLMQKIQRDEDS
	358 20mer	RPCEQHLMQKIQRDEDSYER
	274 10mer	CEALQQIMEN
node93x	131 15mer	RCCNELNEFENNQRC
	132 15mer	CNELNEFENNQRCMC
	204 20mer	SSQHQCRCNELNEFENNQRC
	205 20mer	QHQCRCNELNEFENNQRCM
	206 20mer	QERCCNELNEFENNQRCMCE
	207 20mer	RCCNELNEFENNQRCMCEAL
	208 20mer	CNELNEFENNQRCMCEALQQ
	324 15mer	CCNELNEFENNQRCM
	375 20mer	SQHQCRCNELNEFENNQRC

TABLE E1. Continued

Peptide	Length	Sequence
node156x	138 15mer	CMCEALQQIMENQSD
	139 15mer	CEALQQIMENQSDRL
	212 20mer	NNQRCMCEALQQIMENQSDR
	213 20mer	QRCMCEALQQIMENQSDRLQ
	214 20mer	CMCEALQQIMENQSDRLQGR
	217 20mer	QQIMENQSDRLQGRQQEQF
	328 15mer	RCMCEALQQIMENQS
	329 15mer	CEALQQIMENQSDRL
	379 20mer	EFENNQRCMCEALQQIMENQ
	380 20mer	NNQRCMCEALQQIMENQSDR
	381 20mer	RCMCEALQQIMENQSDRLQ
	382 20mer	CEALQQIMENQSDRLQGRQQ
	384 20mer	IMENQSDRLQGRQQEQFQR
node127x	73 10mer	RNLPPQCGLR
	147 15mer	GRQQEQQFKRELRLN
	150 15mer	QFKRELRLNPPQCGL
	221 20mer	DRLQGRQQEQQFKRELRLNLP
	222 20mer	LQGRQQEQQFKRELRLNPQ
	223 20mer	GRQQEQQFKRELRLNPPQCG
	224 20mer	QQEQQFKRELRLNPPQCGLR
	225 20mer	EQQFKRELRLNPPQCGLRAP
	226 20mer	QFKRELRLNPPQCGLRAPQR
	227 20mer	KRELRLNPPQCGLRAPQRCD
	337 15mer	KRELRLNPPQCGLRA
	386 20mer	DRLQGRQQEQQFKRELRLNLP
	387 20mer	QGRQQEQQFKRELRLNPQC
	388 20mer	QQEQQFKRELRLNPPQCGLR
	389 20mer	QQFKRELRLNPPQCGLRAPQ
	390 20mer	KRELRLNPPQCGLRAPQRCD
	81 10mer	LDVESGGRDR
	82 10mer	DVESGGRDRY
	233 20mer	GLRAPQRCDLDVESGGRDRY
	160 20mer	LTILVALALFLAAHASARQ
	239 10mer	AAHASARQQW
	171 20mer	ELQGDRRCQSQLERANLRPC
node212x	22 10mer	EQHLMQKIQR
	100 15mer	ERANLRPCEQHLMQK
	176 20mer	QLERANLRPCEQHLMQKIQR
	249 10mer	EQHLMQKIQR
	303 15mer	ANLRPCEQHLMQKIQ
	20 10mer	LRPCEQHLMQ
	23 10mer	HLMQKIQRDE
	101 15mer	ANLRPCEQHLMQKIQ
	248 10mer	RPCEQHLMQK
	354 20mer	RRCQSQLERANLRPCEQHLM
	153 15mer	RNLPPQCGLRAPQRC
	157 15mer	LRAPQRCDLDVESGG
	125 15mer	DRRGAGSSQHQRCC
	199 20mer	PSPYDRRGAGSSQHQRCCN
	265 10mer	RRGAGSSQHQ
	167 20mer	HASARQQWELQGDRRCQSQL
	238 10mer	FLAAHASAR
	347 20mer	LALFLAAHASARQQWELQG
	269 10mer	CCNELNEFEN