

Microarray immunoassay: Association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes

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Background: IgE epitope mapping of food allergens is a prerequisite for engineering hypoallergenic immunotherapeutic agents and might reveal basic information regarding a patient's immune response. Mapping of large numbers of epitopes by using individual patient sera has been impractical with current techniques.

Objective: We sought to develop a peptide microarray-based immunoassay to map peanut epitopes by using microliter quantities of serum.

Methods: A set of 213 overlapping 20-residue peptides was synthesized corresponding to the primary sequences of Ara h 1, Ara h 2, and Ara h 3. These were arrayed in triplicate along with the corresponding recombinant proteins onto glass slides and used for immunolabeling.

Results: Seventy-seven patient and 15 control sera were analyzed. The majority of patients (97%) had specific IgE to at least one of the recombinant allergens, and 87% had detectable IgE to sequential epitopes. Microarray mapping correlated well with previous studies. However, the analysis of individual patients revealed remarkable heterogeneity in the number and patterns of epitope recognition. High epitope diversity was found in patients with a history of more severe allergic reactions. Also, sensitization of effector cells with more diverse IgE antibodies conferred greater reactivity to specific allergen. **Conclusions:** The protein microarray immunoassay confirmed that Ara h 1, Ara h 2, and Ara h 3 are major peanut allergens and allows for parallel epitope analysis. This has led to the discovery of an additional important epitope of Ara h 1 and the recognition of a high degree of patient heterogeneity. This qualitative difference in epitope diversity might provide prognostic information about the patient. (*J Allergy Clin Immunol* 2004;113:776-82.)

Key words: Peanut allergy, IgE, protein microarray, peptide microarray, epitope mapping, epitope spreading

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Abbreviations used

HAS: Human serum albumin

MIA: Microarray-based immunoassay

The characterization of IgE epitopes of important food antigens is of fundamental importance for understanding mechanisms of food allergy and for designing safe immunotherapeutics.^{1,2} Although antibodies naturally encounter and are selected for their ability to bind the 3-dimensional surface of an antigen, including epitopes that are not made up of sequential amino acids, identification of primary sequences that are recognized by IgE provides important information regarding a protein's antigenicity. This is especially true for food allergens. We have previously shown that the alteration of Ara h 1—allergenic epitopes identified solely by means of analysis of sequential epitopes results in profound diminution of IgE binding for the majority of patients.³

There also are accumulating data suggesting that differences in epitope recognition might be a useful prognostic marker of a patient's immune response. In the cases of certain milk,^{4,5} egg,⁶ and peanut⁷ allergens, we have shown that individuals with persistent allergy recognize a larger number of sequential epitopes, including some that appear to be specific or informative epitopes, compared with those who outgrow their food allergy. Specific host or stochastic factors might induce certain individuals to generate an immune response that is more broad based or biased toward recognition of certain determinants. Even among patients with persistent allergy, analysis of the patterns of IgE epitope binding might be informative. Little is known about the extent of diversity among individual patients, what might underlie the differences that exist, what consequence it might have on immune function, and how these patterns change over time.

Most of the data regarding IgE epitopes of food proteins to date have been generated through the use of the SPOTS membrane-based immunoassay.⁸ However, for the characterization of large numbers of individual patient allergen-specific IgE antibodies, this method has technical disadvantages. Synthesis of large numbers of peptides is relatively time consuming, labor intensive, expensive, and error prone. Subsequent immunoassays require milliliter quantities of patient serum, and although individual

membranes can be stripped, they can only be reused for a limited number of patients. For these reasons, identification of epitopes is often performed with pooled sera, and evaluation of individual patients has generally been limited. These technical considerations affect the experimental design because it is impractical to assay multiple controls to define positive cutoff values or to perform replicates to determine assay variance.

We report here the development of a microarray-based immunoassay (MIA) that allows parallel analysis of IgE binding to a complete set of overlapping peptides from the sequences of the peanut allergens Ara h 1, Ara h 2, and Ara h 3, as well as the recombinant proteins, by using microliter amounts of patient serum per assay. Using this assay, we have analyzed peanut-sensitized patient serum samples from more than 75 different individuals. The antigenic-allergenic regions identified correlate well with the previously reported results of epitope mapping. However, use of this assay revealed one new major epitope of Ara h 1 and several minor epitopes of Ara h 1, Ara h 2, and Ara h 3. In addition, the degree of heterogeneity among sensitized individuals with respect to their epitope-binding patterns is newly appreciated. Greater diversity of peptide epitope recognition correlates only weakly with peanut-specific IgE levels. However, patients with IgE binding to a greater number of epitopes have a history of more severe peanut-induced allergic reactions. In addition, epitope diversity corresponds with functional capacity to induce activation of FcεRI-bearing cells in response to peanut allergen but not to anti-IgE.

METHODS

Patient sera

Patient and control sera were selected retrospectively on the basis of detectable IgE to peanut, as measured by using the Pharmacia CAP System FEIA. We recruited patients from the Mount Sinai Allergy clinic and obtained detailed food reaction histories at that time. Medical records were reviewed for history of reaction frequency and symptoms. Reaction severity was also ranked on a scale of 0 to 6, as previously described.⁹ The age of peanut-sensitized individuals at the time of recruitment ranged from 1.5 to 36 years (median, 6.9 years; $n = 57$). Peanut-specific IgE ranged from 1.97 to greater than 100 kU/L (median, >100). Of these patients, 38 had a history of positive double-blind, placebo-controlled food challenge results or a convincing history of a clinical reaction. The remaining 18 had increased IgE levels predictive of clinical reactivity (>15 kU_A/L; median, >100) but with no history of known ingestion. Additional sera from patients with confirmed clinical peanut allergy ($n = 20$) were obtained from the University of Arkansas allergy practice and have been previously described.³ Control sera were from patients not allergic to peanut recruited from the Mount Sinai Pediatric Allergy Clinic ($n = 10$) or from healthy volunteers ($n = 5$). All subjects provided informed consent, and all protocols received local institutional review board approval.

Microarray

A set of 20-amino-acid peptides (20-mers) with 15-amino-acid overlap were commercially synthesized and stored in 10 mg/mL PBS at -70°C . These were diluted to 0.3 mg/mL in PBS-30% glycerol and spotted in triplicate on poly-L-lysine-coated microscope slides (Electron Microscopy Sciences) 375 nm apart by using a 4-pin GMS

417 arrayer (Affymetrix). Two peptides derived from Ara h 3 sequences 146 to 165 and 151 to 175 (sequence from Rabjohn, 1999¹⁰) were excluded from printing because they were insoluble. Four grids were created within a less than $22 \times 22\text{-mm}^2$ area. These were allowed to air-dry and stored in a desiccator at room temperature until used.

Immunoassay

All incubations were carried out under a standard $22 \times 22\text{-mm}^2$ cover slip (Corning) in a humidity chamber (The Binding Site). An area just larger than a cover slip around the array was outlined on all 4 sides with a PAP pen. Slides were preincubated with 80 μL of PBS with 0.1% Tween 20 (PBS-T) containing 1% human serum albumin (PBS-T-HSA) for 60 minutes at 23°C . The slides were rinsed 5 times in PBS-T, and 80 μL of human serum diluted 1:5 in PBS-T-HSA was incubated for 30 minutes at 23°C . After rinsing with PBS-T, 80 μL of polyclonal rabbit anti-human IgE (DAKO) diluted 1:500 in PBS-T-HSA was incubated for 30 minutes at 23°C . Slides were then rinsed 5 times with PBS-T and incubated with 150 μL of biotinylated anti-rabbit IgG (Link, DAKO). Slides are again rinsed and incubated with 100 μL of Avidin Cy3 (Amersham) diluted 1:1000 in PBS-T for 30 minutes at 23°C . Slides were finally rinsed 5 times with PBS-T and then dH_2O and centrifuged to dryness (1000g in empty 50-mL conical tubes). Fluorescence emission was measured at 550 nm by using a GMS 418 Array Scanner (Affymetrix). One peptide from Ara h 2, residues 90 to 109, was excluded from analysis because of nonspecific binding of the secondary antibody.

Analysis

Fluorescence intensity was digitized by using ScanAlyze (M. Eisen, <http://rana.lbl.gov/EisenSoftware.htm>) and exported as tab-delimited text. Raw data files were imported into an MS Access/VBA (Microsoft Corp) application developed by our laboratory to relate the data to the appropriate peptide identity. Data were then exported to MS Excel (Microsoft), and the mean intensity of intra-assay triplicates for each peptide was plotted against its coefficient of variation to identify poor-quality data, which were excluded. Each slide included negative (HSA) and positive (a nonspecific biotinylated protein) controls. Relative signal from each peptide was defined as the mean replicate value divided by the positive control mean replicate value.

For interassay comparison, the intra-assay values were $\log_{10}$ transformed for normalization and improvement of variance homogeneity and then adjusted to set the median from each slide equal to zero. A set of samples from 15 donors without peanut allergy, including both nonatopic ($n = 5$) and atopic ($n = 10$) individuals, were selected to define positive levels of interassay IgE binding. The interassay peptide cutoff point was defined as the mean plus 2 SDs of signal from this control group. For the purpose of this study, the cutoff point for positive binding was set at the larger of either the interassay individual peptide cutoff or the intra-assay cutoff (mean plus 2 SDs for all peptides, usually about 0.2).

Basophil assay

Basophils were enriched together with PBMCs from healthy donors by means of Ficoll separation of blood diluted 1:1 with PBS. Washed cells were incubated overnight in complete medium with or without patient serum containing peanut-specific IgE. Cells were then washed twice, and 100 μL per well of cell suspension at a concentration of $10^6/\text{mL}$ was distributed to 96-well, flat-bottom, tissue-culture plates in serum free medium (AIM-V) containing IL-3 (2 ng/mL; R&D Systems, Inc). Antigens were serially diluted at $2 \times$ final concentrations in AIM-V plus IL-3 (2 ng/mL), and 100 μL was added to the wells and allowed to incubate at 37°C and 5% CO_2 for 30 minutes. Negative and positive control wells contained medium alone

TABLE I. Patient IgE recognition of recombinant peanut allergens determined by means of MIA

Allergen	Binding pattern							Percent positive*
Ara h 1	X	X		X	X			77
Ara h 2	X		X	X		X		75
Ara h 3	X	X	X				X	77
Percent positive*	50.6	10.4	10.4	9.1	6.5	5.2	5.2	97.4†

*n = 77.

†Sum of row (ie, percent positive to at least one protein).

or anti-IgE (0.5 µg/mL), respectively. Degranulation was measured by means of flow cytometry as the percentage of CD203c-positive cells expressing high levels of CD63.^{11,12} Briefly, after activation, cells were harvested and washed in PBS containing 2 mmol/L EDTA and 1% BSA (PBS-EDTA-BSA). Cells were then resuspended (approximately 8×10^4) in 50 µL of PBS-EDTA-BSA and stained with previously titrated amounts of anti-CD63 FITC (clone H5C6, BD) and anti-CD203c PE (clone 97A6, Beckman Coulter). Unstained, compensation, and isotype controls were also prepared from the same donor cells. At least 50,000 events for each sample were acquired on a FACSCalibur (BD), and data were compensated and analyzed by using FlowJo (Tree Star).

RBL assay

The human FcεRI-transfected rat basophilic leukemia cell line RBL-SX38 (kind gift of J. P. Kinet, Harvard University) was cultured in the presence of 1.0 mg/mL G418 (Invitrogen), as previously described.¹³ Before reaching confluence, cells were harvested, washed, and counted. In a total of 100 µL of complete medium, 10^6 cells were sensitized with saturating concentrations of human serum for 1 hour at 37°C. These were then washed and diluted to 10^4 /mL, and from this, 200 µL was distributed per well to 96-well, flat-bottom, tissue-culture plates. After incubating for 2 hours at 37°C to allow cells to adhere, the medium was replaced with Eagles' minimal essential medium containing serially diluted antigen. Control wells were replaced with Eagles' minimal essential medium alone or wells containing anti-IgE (0.5 µg/mL). Cells were incubated for 30 minutes at 37°C. Supernatants were analyzed for histamine content by using an automated fluorescence-based assay (Astoria Micro Flow Analyzer, Astoria Pacific Int).

Statistics

Patient versus control IgE mean binding to recombinant allergens was compared by using unpaired *t* tests, and reported *P* values are 2-tailed. The Mann-Whitney *U* test was used to analyze differences in epitope diversity between patient groups.

RESULTS

Assay characteristics

We performed replicate experiments by using a pool of sera from patients with peanut allergy to investigate interassay and intra-assay reproducibility. Each array also included whole recombinant Ara h 1, Ara h 2, and Ara h 3. The same pooled sera were run on 4 separate arrays. The intra-assay coefficient of correlation for any one of the 4 assays was greater than 0.95, and the variance was homogenous. The interassay mean coefficient of variation for peptide binding was 3.4%, with a range of 0.1% to 14%, which correlated with intensity of staining.

Recognition of whole recombinant Ara h 1, Ara h 2, and Ara h 3

Ara h 1, Ara h 2, and Ara h 3 have been previously described as major peanut allergens.^{10,14,15} The MIA allowed us to examine the prevalence of IgE recognition of these allergens for a large number of patients (n = 77). Consistent with previous results, the majority of sera from peanut-sensitized patients contained IgE to these recombinant proteins (Table I). We found that 97% of these patient sera contained specific IgE to at least one of the 3 allergens, whereas 77%, 75%, and 77% recognized Ara h 1, Ara h 2, and Ara h 3, respectively. The comparison of binding between patients and control subjects is shown in Fig 1. The mean signal ratio for Ara h 1 was 0.72 for patient sera versus 0.14 for sera from control subjects not allergic to peanut (95% confidence range, 0.38-0.78; *P* < .0001). For Ara h 2 and Ara h 3, the respective means were 0.77 (95% confidence range, 0.42-0.88) and 0.86 (95% confidence range, 0.64-0.90; *P* < .0001 for each). There was no significant difference when individuals with confirmed clinical peanut allergy were compared with those with predictive peanut-specific IgE levels but unproved clinical status (not shown).

Antigenic areas identified with the MIA correspond well with previously identified regions

Fig 2, A, is a bar graph showing the frequency of patient serum samples with positive IgE binding to each peptide displayed on this array for 77 patients. Peptides are ordered on the x-axis from N- to C-termini of each of the 3 allergens (Ara h 1 in red, Ara h 2 in green, and Ara h 3 in blue). The dark bars represent location of the epitopes previously defined by SPOTS membrane mapping.^{10,16,17} Thirty-nine peptides defining 21 major antigenic regions are recognized by more than 5 patients. Ara h 1 contains 13 prominent antigenic regions, whereas Ara h 2 has 4 and N-Ara h 3 also has 4. No single peptide was recognized by more than 35% of the samples tested. However, 67 (87%) of 77 of these samples were positive to at least one peptide. The mean number of peptides recognized was 9.4, and the median was 5, with a range of 0 to 56.

As can be seen, there is substantial agreement between the 2 methods in the identification of IgE-binding regions. The use of 20-mers produces a lower-resolution map; however, all of the regions of Ara h 1 and Ara h 2 previously identified are recognized by using the microarray immunoassay as well. This is not the case for Ara h 3, for which very few individual sera of these patients recognized the first previously identified epitope. In addition, no single epitope is recognized by more than 35% of the patients on this platform, a striking difference from what has been published with SPOTS analysis, which identified single epitopes recognized by the majority of individual samples assayed, albeit with significantly fewer patients analyzed.^{7,10,16,17}

One region of Ara h 1 represented by peptides 73 and 74, corresponding to residues 361 to 385, showed

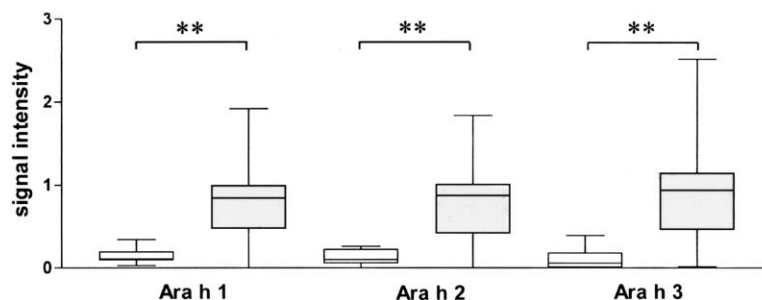


FIG 1. The microarray immunoassay detects allergen-specific IgE. Peanut-sensitized patients ($n = 77$, shaded boxes) versus control subjects ($n = 15$, open boxes) have significantly higher levels of IgE, as detected by using this assay to rAra h 1, rAra h 2, and rAra h 3 (** $P < .001$).

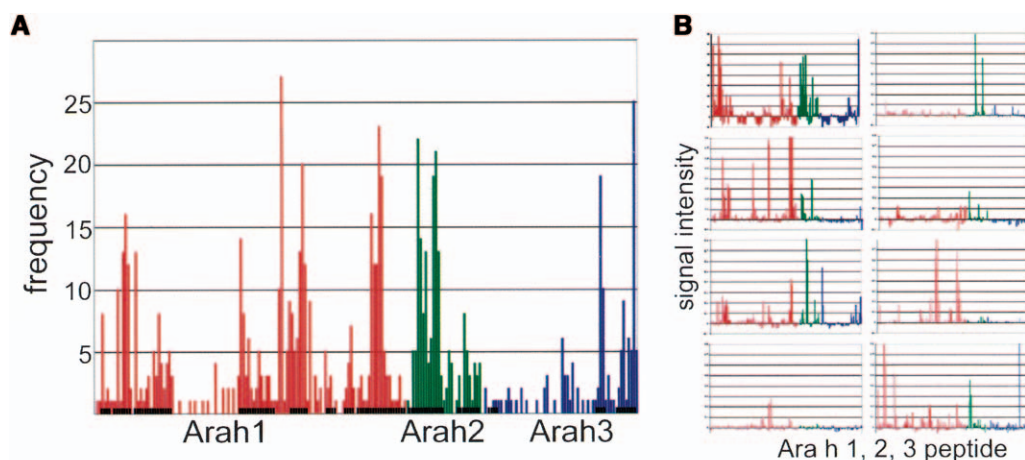


FIG 2. IgE epitope profiles. **A**, The frequency of recognition plotted against each peptide on the microarray ordered from N to C terminus of Ara h 1 (red), Ara h 2 (green), and Ara h 3 (blue; $n = 77$). **B**, Eight individual patient IgE profiles.

significant IgE binding by using the microarray assay. Identification of this epitope suggested that this region might have been missed in previous analyses by virtue of the fact that initial mapping was carried out with pooled sera. We therefore reanalyzed this region by means of SPOTS membrane immunoassay with these and other individual sera identified as recognizing peptides 73 and 74. These patients also recognize this region by means of SPOTS analysis, confirming the result obtained with MIA and underscoring the advantages of using individual sera for epitope mapping (data not shown).

Individual patients have heterogeneous patterns of IgE binding

Perhaps the most striking feature of IgE binding revealed by means of MIA is the variability from patient to patient with respect to both the number of peptide epitopes recognized and the pattern of recognition. Fig 2, B, shows 8 representative graphs, plotting signal against peptide, that demonstrate this large degree of heterogeneity.

IgE diversity corresponds to a history of reaction severity

Food antigen-specific IgE concentrations alone have been shown to have prognostic value for likelihood of

reactivity.^{18,19} For the patients in this study, who as a group have high peanut-specific IgE levels (median, >100 kU/L), there was no significant correlation between IgE and historical reactivity (not shown). We hypothesized that greater epitope diversity might be a marker for more severe reactions.

Patients were divided into those with cutaneous symptoms alone ($n = 14$) versus those with multiple organ system involvement ($n = 24$). The patients with a history of more severe multisystem reactions recognized a significantly greater number of epitopes than those with a history of cutaneous symptoms alone (median, 9.5 vs 2, respectively; $P < .01$; Fig 3, A). The same patients have similar recognition of whole recombinant peanut allergens (Fig 3, B).

In addition, when all peanut-sensitized patients were segregated by median number of positive epitopes recognized (5 epitopes), those with greater epitope diversity had a history of significantly more severe reactions. For the low-diversity group, the 95% CI for reaction score was 0.67 to 1.5, whereas for the high-diversity group, the interval was 1.3 to 2.2 ($P < .05$; $n = 27$ for each group). This is not the case when these patients were segregated on the basis of the quantity of recombinant peanut protein-specific antibody or CAP-

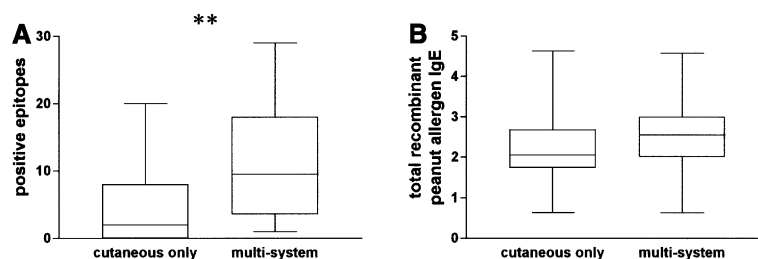


FIG 3. Individuals with a history of more severe peanut-induced reactions recognize more peanut allergen epitopes. **A**, Individuals with a history of cutaneous symptoms ($n = 14$) recognize fewer epitopes (median, 2; range, 0-20) compared with those with a history of reactions involving multiple organ systems ($n = 24$; median, 9.5; range, 1-29; $P < .01$). **B**, The same patients have similar recognition of recombinant peanut allergens.

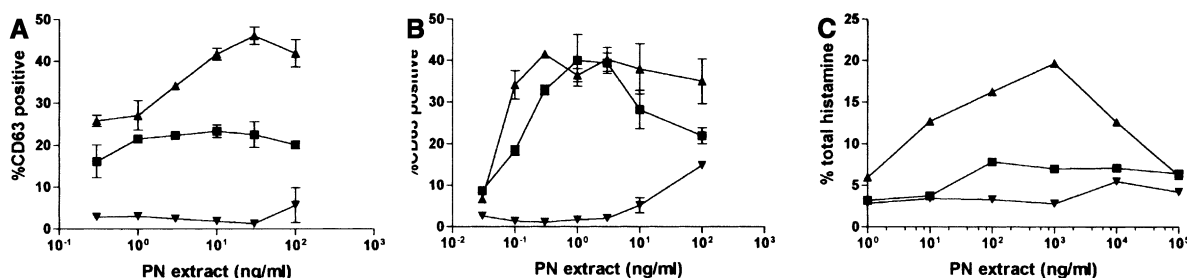


FIG 4. Patient sera recognizing a greater number of IgE epitopes induce more degranulation of passively sensitized cells. Pooled sera from patients with high levels of peanut-specific IgE (>100 kU/L; $n = 4$ for each group) were generated from sera recognizing few epitopes (*squares*) versus sera recognizing many epitopes (*triangles*) and sera from control subjects not allergic to peanut (*diamonds*). Serum pools were used to passively sensitize human basophils from a nonatopic donor (**A**), an atopic donor not allergic to peanut (**B**), and the rat basophilic leukemia cell line expressing the human Fc ϵ RI (**C**). PE, Peanut.

FEIA peanut-specific IgE (not shown). A possibly related trend, which failed to reach statistical significance, was seen with respect to the number of documented reactions. The high-diversity group had a median of 1.5 reactions (range, 0-6) versus the low-diversity group, with a median of 0 reactions (range, 0-3; $P = .08$). In comparison, when segregated on the basis of median recombinant peanut allergen binding, both groups had a similar number of reactions (median, 1; $P = .96$).

IgE diversity correlates with effector function in vitro

The differences found among the patients' histories of reactions suggested that epitope diversity might be related to IgE function. To test this, we adapted 2 bioassays of IgE function. In the first set of experiments, basophils from donors not allergic to peanut were incubated with pooled sera from individuals with high- or low-diversity peanut-specific IgE ($n = 4$; median IgE, >100 kU/L for each group). These pooled sera were matched for ratio of peanut-specific to total IgE and used at equal concentrations. After sensitization, cells were stimulated with peanut extract, and degranulation was measured as increased surface expression of CD63.²⁰⁻²³ Fig 4 shows representative results from one passively sensitized normal nonatopic donor (Fig 4, A) and one atopic donor

without peanut allergy (Fig 4, B). In both cases the high-diversity IgE sera conferred significantly greater sensitivity to peanut extract ($P < .001$ and $P < .05$, respectively). The greater response was specific to peanut allergen; no difference in the response to anti-IgE was seen for either donor (not shown). To further test the functional capacity of these 2 pooled sera, we used the rat basophilic leukemia cell line expressing human Fc ϵ RI (RBL-SX38, kindly provided by J. P. Kinet).^{13,24} Consistent with our hypothesis and the results with passively sensitized human basophils, the more diverse IgE-containing sera conferred greater effector response ($P < .001$; Fig 4, C).

DISCUSSION

With the largest patient sample to date, we have confirmed the allergenicity of Ara h 1, Ara h 2, and Ara h 3. By using this novel assay, our findings are consistent with those of previous reports on the prevalence of IgE to these allergens. Kleber-Janke et al²⁵ investigated the prevalence of IgE binding by means of immunoblotting to a number of recombinant allergens from peanut, including Ara h 1 and Ara h 2. Using Western blots, they found that 65% and 85% of 40 patients' sera IgE bound Ara h 1 and Ara h 2, respectively. In addition, they showed that 53% of their patients had demonstrable IgE to Ara h 4, which has

91% amino acid identity to Ara h 3. Similar to our findings of patients' IgE binding to Ara h 1, Ara h 2, and Ara h 3 (Table I), they demonstrated that approximately half of their patients' sera (18/40) recognized all 3 allergens. In the case of Ara h 1, investigators have reported prevalence of specific IgE among patients ranging from less than 50% to greater than 90%.^{26,27} For Ara h 2, most studies have found IgE recognition in more than 70% of patients.¹⁵ Many studies have relied on relatively few patient sera. Kleber-Janke's study was previously the largest. Our results with 77 patient sera confirm the importance of these allergens.

One obvious goal of IgE epitope mapping is the development of engineered allergens that have reduced IgE binding and therefore a larger therapeutic window for use in immunotherapy. This effort is already well under way.^{1,2} However, we are also interested in what information might be derived regarding a patient's immune response. In the context of autoimmunity or infection, epitope spreading or diversification is a hallmark of immune progression. This regulated process goes hand in hand with affinity maturation and reflects a T-dependent process of activation, expansion, and differentiation of reactive B cells after antigen exposure.^{28,29} In the context of food allergy, epitope diversification has not been rigorously demonstrated.³⁰ However, greater antibody diversity has been identified with stronger or more persistent immune responses. For example, we found that patients with egg allergy, compared with control subjects, recognized greater numbers of IgG epitopes of ovomucoid.⁶ Similarly, others have found a greater diversity of IgG recognition patterns to peanut allergens in patients compared with control subjects by means of immunoblotting, despite the lack of difference in levels of specific IgG.³¹ In addition, patients allergic to milk, egg, and peanut with persistent compared with transient allergy have been found to recognize a greater number of IgE epitopes.⁴⁻⁷

It is possible that epitope analysis might be useful for predicting the natural history of a patient's allergy. Prospective studies monitoring IgE and IgG epitope patterns will address this potential. In preliminary results we have found that peanut allergen-specific IgG epitope diversity correlates well with IgE diversity (Shreffler, unpublished data). Epitope diversity is expected to be both reflective of immune progression and predictive of antibody effector function. The in vitro data presented here do support the prediction that antibodies recognizing a greater number of determinants more efficiently induce effector cell responses. This difference is likely to extend to other antibody functions, such as enhanced antigen capture, focusing, and presentation to the adaptive immune system.

Microarray technology is well suited to monitor changes in epitope recognition of several allergens simultaneously by using small amounts of patient sera.^{32,33} This is the first application of this technology for the purpose of allergen epitope mapping of which we are aware. Hiller et al³⁴ recently demonstrated the

suitability of this technique for the surveillance of IgE to multiple whole allergens. We have also included whole allergens in our assay and demonstrated specific recognition of these allergens by patients. Although the sensitivity of MIA has not been directly compared with that of other methods, the percentage of patients recognizing these allergens with this method is well within the range of what has been reported in the literature. Furthermore, specific IgE antibodies to both whole recombinant allergen, as well as specific peptides, have been detected in patients with CAP-FEIA IgE to peanut ranging down to less than 2 kU_A/L.

The advantages of the microarray assay allow for the characterization of large numbers of individual patient samples. This approach has led to the identification of an additional Ara h 1 epitope previously missed by using pooled sera, despite the fact that 3 of the original 12 samples used for mapping contained IgE specific to this epitope.

This approach also underscores the diversity of patient IgE responses to allergen. In addition to the magnitude of sequential epitope recognition, we also have performed preliminary analyses of recognition patterns by using hierarchic clustering of array data. Included in this analysis were a total of 90 serum samples from 83 individuals, including some subjects with low levels of peanut-specific IgE without a history of reactivity or with a history of outgrowing their peanut allergy, and multiple samples from 4 individuals at different time points. Of note, all 15 control subjects without peanut allergy, including atopic subjects, subjects with food allergy, and nonatopic subjects, cluster into a single group. In addition, multiple samples of the same patient over time cluster significantly (Shreffler, unpublished data). These patient groups, however, did not correlate with historical reactivity or any other clinical parameter analyzed.

A better understanding of epitope diversity and its progression or regulation should lead to improved therapeutic strategies and better monitoring of immunotherapeutic interventions. Prospective studies addressing the phenomenon of determinant spreading and its potential relationship to allergy persistence or tolerance will be informative. The microarray immunoassay makes such large-scale epitope surveillance studies practical.

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