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IgE binding to linear epitopes of Ara h 2 in peanut allergic preschool children undergoing oral Immunotherapy

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Abstract

Background: For patients with peanut allergy, there are currently no methods to predict who will develop sustained unresponsiveness (SU) after oral immunotherapy (OIT).

Objective: Assess IgE binding to peanut (PN), Ara h 2, and specific linear epitopes of Ara h 2 as predictors of the important clinical parameters: eliciting dose threshold and attainment of SU following OIT.

Methods: Samples and clinical data were collected from children undergoing OIT. PN- and Ara h 2-sIgE were quantified by ImmunoCAP[®]. IgE binding to linear peptides of Ara h 2 and Ara h 6 was measured with peptide microarrays.

Results: Values of PN-sIgE correlated with eliciting dose ($P = .001$) and with a higher likelihood of achieving SU ($P < .0001$), but these relationships were lost at higher values for PN-sIgE (≥ 14 kIU for eliciting dose and ≥ 35 kIU/L for SU). In subjects with PN-sIgE ≥ 14 kIU/L, binding of IgE to epitopes 5 and 6 of Ara h 2 was associated with a lower eliciting dose at baseline challenge ($P < .001$; $P_c < .02$). In subjects with PN-sIgE ≥ 35 kIU/L, a combined model of IgE binding to epitopes 1, 5, and 6 with PN-sIgE was highly predictive of attainment of SU (AUC of 0.86; $P = .0067$).

Conclusion: In young patients with peanut allergy, measurement of PN-sIgE and IgE binding to specific linear epitopes of Ara h 2 in baseline samples may allow stratification of patients regarding sensitivity to challenge and outcome of OIT.

KEYWORDS

allergens, food allergy, IgE, immunotherapy, oral immunotherapy, peanut allergy, tolerance induction

1 | INTRODUCTION

IgE-mediated food allergy, particularly to peanuts (PN), is a major health problem, affecting 1%-4% of children in the United States and northern Europe.¹⁻⁴ Oral immunotherapy (OIT) is generally associated with favorable outcomes including attainment of sustained unresponsiveness (SU).⁵⁻⁹ SU is most likely to occur in subjects who start with lower levels of peanut-sIgE and particularly in subjects who begin OIT at a younger age.¹⁰ Although the basophil activation test correlates with severity and threshold of allergic reactions to PN,^{11,12} additional biomarkers, particularly that do not need live cells, are needed to predict sensitivity to challenge and new biomarkers are needed to predict who will develop SU after OIT.

Among the 17 known PN allergens,¹³ the highly homologous 2S albumins, Ara h 2 and Ara h 6, are the most potent for eliciting IgE-mediated mast cell activation.¹⁴⁻¹⁷ Measurement of specific IgE to either Ara h 2 or Ara h 6 has higher diagnostic value for predicting clinically relevant PN allergy than does measurement of IgE to other peanut proteins.^{18,19}

Patterns of IgE binding to linear epitopes of peanut allergens in microarray immunoassays have described both positive²⁰⁻²² and negative²³ correlations between the complexity of epitopes identified and the presence of clinically relevant peanut allergy.

This study was designed to explore, in a cohort of children undergoing OIT, possible associations of PN- or Ara h 2-sIgE and/or of IgE binding to linear epitopes of Ara h 2 and Ara h 6 with either sensitivity to peanut allergens during challenge or the attainment of sustained unresponsiveness (SU) as described in the next section.

2 | METHODS

2.1 | Subjects and samples

Samples of plasma were obtained from a study of OIT in preschool children with suspected or known peanut allergy, the DEVIL study.¹⁰ The analysis of these samples at the University of Colorado, Denver, was approved by the respective institutional review boards, and either the parents or guardians for all subjects gave informed consent. Single aliquots of plasma, obtained prior to challenge and OIT, were thawed once and refrozen in aliquots at -80°C.

The DEVIL study enrolled a total of 49 subjects, 9-36 months of age who were peanut-allergic or peanut-sensitized (Table S1). The first 40 subjects enrolled in the study were previously reported¹⁰ as that was the sample size required by the power analysis. Of the 49 subjects enrolled, 46 reacted with clinically significant signs and symptoms to an open-label challenge at doses ranging from 1 to 300 mg of peanut protein (the eliciting dose). For those successfully completing OIT, peanuts were withheld for 4 weeks and a final double-blind placebo-controlled challenge was performed. SU was defined as lack of reactivity to a challenge with 5000 mg of peanuts at this final challenge. Those who either withdrew or completed the study and did not achieve SU were grouped together as not achieving SU.

Key message

For patients with peanut allergy, there are currently no methods to determine who will develop sustained unresponsiveness (SU) after oral immunotherapy (OIT). Our objective was to assess IgE binding to peanut (PN), Ara h 2 and to specific linear epitopes of Ara h 2 as predictors of the important clinical parameters: eliciting dose threshold and attainment of SU following OIT. We report that, in young patients with peanut allergy undergoing OIT, measurement of both PN-sIgE and IgE binding to specific linear epitopes of Ara h 2 in baseline samples may allow stratification of patients regarding sensitivity to challenge and outcome of OIT.

Measurement of IgE binding to specific linear epitopes of Ara h 2 and Ara h 6 was limited to samples with PN-IgE ≥ 14 IU/mL, a criterion for our previous study that allowed optimal detection of IgE binding to epitopes²³ and, at least for older children, identifies a group of patients with a high likelihood of having a positive PN challenge.²⁴ Of the 46 subjects enrolled, 27 had baseline PN-sIgE ≥ 14 kIU/L. Four samples were not studied due to insufficient numbers of microarrays, leaving 23 samples that were analyzed in this study (Table S2).

2.2 | Peptides and microarray printing

A library of 77 peptides, consisting of 20 amino acids (AAs) in length overlapping by 17 AAs (offset of 3) covering the entire sequence of Ara h 2 and its isoforms, and Ara h 6 and its isoforms, was synthesized as previously described.²³

2.3 | Assays

Peanut-specific IgE (PN-sIgE), peanut-specific IgG₄ (PN-sIgG₄), and Ara h 2-specific IgE (Ara h 2-sIgE) were determined by ImmunoCAP®. For the microarray assays, all samples of plasma were assayed as previously described.²³ Each sample was studied 2-3 times on separate days, and the data were averaged. Within-assay coefficient of variance (CV) ranged from 16% to 22% with a mean of $18 \pm 2\%$. Between-assay CV was determined for three sera that were assayed on 3 - 11 separate days. For peptides that were part of epitopes that had a Z-score > 3 (see below), the between-assay CV ranged from 17% to 61% with a mean of $39 \pm 2\%$.

2.4 | Statistics

R statistical software²⁵ and GraphPad Prism 5.0c for the Macintosh (GraphPad) were used to generate graphs and for statistical analysis. For analysis of sIgE levels (PN, Ara h 2, and specific peptides) and for eliciting dose, the data were log₁₀-transformed prior to statistical analysis. The following statistical tests were

used: Spearman's rank-order correlation coefficients for correlations; Student's *t* test for comparison of geometric means of the log-transformed continuous variables; and Fisher's exact test for comparing frequencies of two possible outcomes. We assessed binding of IgE to 77 overlapping peptides (as described above) and quantified binding to the 15 epitopes that were previously identified from IgE binding to these peptides.²³ So, where applicable, we used a Bonferroni correction of 15. Corrected *P* values are denoted as *P_c*. Once we identified specific epitopes of importance (epitopes 1, 5, and 6 of Ara h 2; see below), we no longer did multiple comparisons. *P* values of $\leq .05$ were considered to be significant. All comparisons except the receiver operating curve (ROC) analyses were two-sided. The ROC curves were generated with R using the ggplot2 package²⁶ and were based on logistic regression models. All AUC data were compared to an AUC of 0.5 using a one-sided Wilcoxon test for which *P* < .025 denotes a significant difference.

Z-scores for individual peptides were calculated similarly to what we previously described.²³ First, measurements for each sample run of different slides were quantile-normalized to reduce interassay variability. Next, we let $Z = \frac{\log_2(m(S_p)/m(\text{empties}))}{\text{mad}(\log_2(\frac{\text{empties}}{m(\text{empties})}))}$, where *m*() denotes the median, and mad denotes the median absolute deviation calculated using the mad function in the R stats, each package²⁵ with default values. This formula is our preference as to how to state the calculation of Z-scores and gives the same results as the formula described previously.²³ Binding to an individual peptide was considered to be positive if the Z-score was >3 (signal was significantly (*P* < .003) above the background).

2.5 | Definition of an epitope and of positive binding of IgE to an epitope

Epitopes were defined as previously described²³ except that, on 4 occasions (out of a total of 345), we elected to call positive binding where there was a very focused immune response for an epitope such that there were significant (Z-score > 3) binding to several peptides within an epitope but the mean Z-score for the previously determined epitopes was <3.

3 | RESULTS

3.1 | Eliciting dose

In subjects enrolled in the DEVIL study, there was an inverse relationship between the dose of peanut protein that elicited symptoms and levels of PN-sIgE (*r* = −.479, *P* = .001) (Figure 1A). The relationship between PN-sIgE and eliciting dose was also seen for subjects with PN-sIgE < 14 kIU/L (*r* = −.460; *P* = .048) (Figure 1B) but not for those with PN-sIgE ≥ 14 kIU/L (*r* = −.103; *P* = .610) (Figure 1C). There was also an inverse relationship between the eliciting dose and levels of Ara h 2-sIgE (*r* = −.457; *P* = .005) (Figure 1D), but this did not hold up if the total cohort was divided into those with PN-sIgE < 14 kIU/L

(*r* = −.132; *P* = .512) (Figure 1E) and those with PN-sIgE ≥ 14 kIU/L (*r* = .105; *P* = .675) (Figure 1F). We also examined levels of PN-sIgG₄. There was no relationship between levels of PN-sIgG₄ and eliciting dose (*r* = −.071; *P* = .637; data not shown). The ratio of PN-sIgE/IgG₄ did correlate with eliciting dose (*r* = −.534; *P* < .0001; data not shown), but this was not substantially different from the data with PN-IgE alone.

We then examined the IgE binding to individual epitopes of Ara h 2 and Ara h 6 in samples with PN-sIgE ≥ 14 kIU/L as described in the Methods section. Prior to correction for multiple comparisons, there was a suggestion of a relationship between binding to epitope 5 (*n* = 3; *P* = .0975; *P_c* = 1) (not shown) and epitope 6 (*n* = 4; *P* = .0128; *P_c* = .192) (not shown) of Ara h 2 and the eliciting dose. However, when binding to either epitope 5 or epitope 6 (*n* = 5) was examined, there was a much stronger relationship with lower eliciting dose. Those subjects whose IgE bound either of these epitopes were more likely to have a lower eliciting dose (*P* < .0005; *P_c* = .008) (Figure 2A). However, the strength of the signals (epitopes 5 + epitope 6 combined) showed only a weak inverse correlation with the eliciting dose (*r* = −.431; *P* = .04) (Figure 2B).

3.2 | Attainment of SU

As was initially reported with the first 40 subjects from the DEVIL study,¹⁰ we found a strong inverse relationship between both PN-sIgE (Figure 3A) and Ara h 2-sIgE (Figure 3B) and achievement of SU (*P* < .0001 for each). We then examined our data for possible cutoff values. Visual examination of the data for those who did not achieve SU (squares in Figure 3) suggested a demarcation at PN-sIgE of 35 kIU/L (dashed line, Figure 3A) and Ara h 2-sIgE of 24 kIU/L (dashed line, Figure 3B). Those achieving SU were much more likely to have PN-sIgE < 35 kIU/L (25 of 32 = 78%) than those not achieving SU (2 of 14 = 14%) (*P* < .0001). Also, those achieving SU were much more likely to have Ara h 2-sIgE < 24 kIU/L (27 of 31 = 87%) than those not achieving SU (2 of 14 = 14%) (*P* < .0001). Looking at these data from the standpoint of these cutoff values, 25 of 27 (93%) of those with PN-sIgE < 35 kIU/L and 25 of 27 (93%) of those with Ara h 2-sIgE < 24 kIU/L achieved SU, whereas, for those with PN-sIgE ≥ 35 kIU/L, only 7 of 20 (35%) achieved SU and, for those with Ara h 2-sIgE ≥ 24 kIU/L, only 4 of 16 (25%) achieved SU (Figure 3A,B). Specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) for these cutoffs are shown in Table S3. So, for subjects with PN-sIgE < 35 kIU/L or Ara h 2-sIgE < 24 kIU/L, there is a strong relationship with attainment of SU (PPV = 0.93). However, for those with PN-sIgE ≥ 35 kIU/L, there was not a clear relationship between the level of either PN-sIgE (Figure 4A) or Ara h 2-sIgE (Figure 4B) and attainment of SU. Of note, there were no significant differences in PN-sIgE, Ara h 2-sIgE, or IgE binding to epitopes between those who withdrew and those who completed the study but did not achieve SU (Figures 3 and 4). We then asked if the frequency or strength of binding of IgE to epitopes of Ara h 2 or Ara h 6 was related to the outcome of SU in this group. Of the 15 epitopes examined, IgE binding only to epitope 1 of Ara h 2 showed a possible

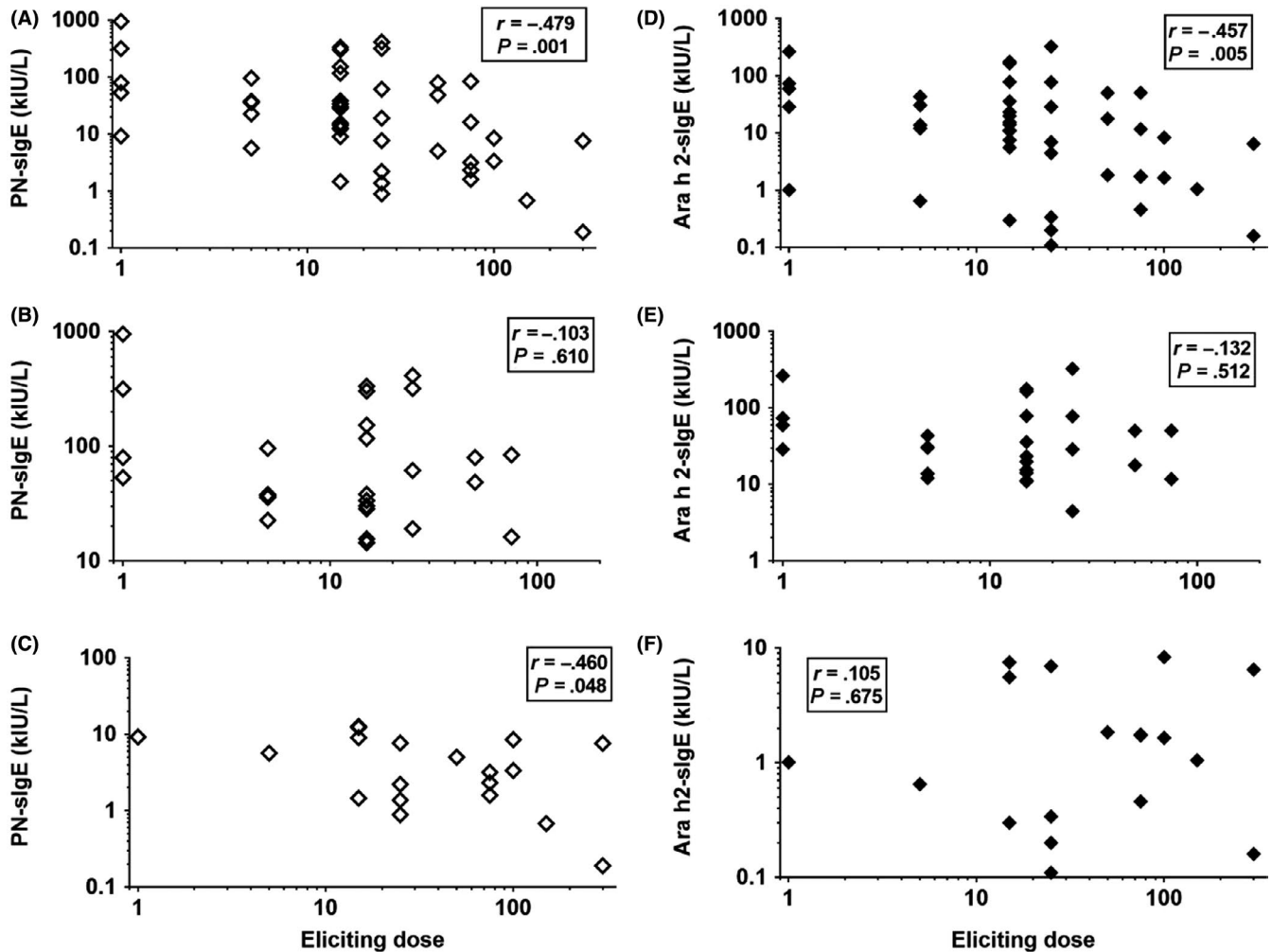


FIGURE 1 Increased levels of PN-sIgE are associated with lower eliciting dose upon oral challenge (A–C). This is true for the entire cohort (A) and for those with PN-sIgE < 14 kIU/L (B). However, this is not seen in those with PN-sIgE ≥ 14 kIU/L (C). Increased levels of Ara h 2-sIgE are also associated with lower eliciting dose upon oral challenge, but only for the entire cohort (D). This relationship was not seen in the subgroups with PN-sIgE < 14 kIU/L or with PN-sIgE ≥ 14 kIU/L (E, F). Data shown are for those who reacted to the initial challenge in the DEVIL study ($n = 46$) (A, D) and for those who reacted and had PN-sIgE < 14 kIU/L (B, E) and those with PN-sIgE ≥ 14 kIU/L (C, F) (see Table S1)

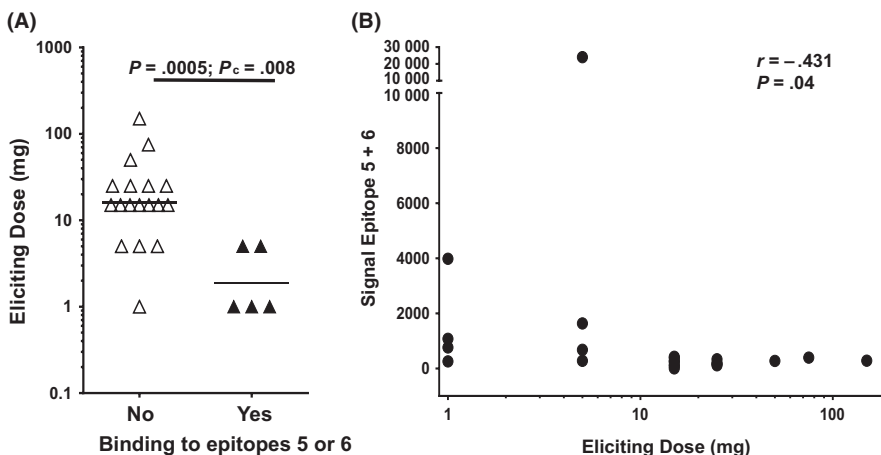


FIGURE 2 IgE binding to epitopes 5 and 6 is associated with lower eliciting dose upon oral challenge. A, Subjects ($n = 23$; all with PN-sIgE ≥ 14 kIU/L) were separated into those that did not have strong evidence of binding to either epitope 5 or epitope 6 ($z < 3$; open triangles) and those that had strong evidence of binding ($z \geq 3$; closed triangles) (see Table S2). B, The raw data (signal from epitope 5 plus the signal from epitope 6) are plotted against the eliciting dose

inverse relationship to achievement of SU (Figure 4C; $P = .0189$; $P_c = .284$).

Finally, we examined the possibility that measuring IgE binding to epitopes of Ara h 2 may improve prediction of attainment of SU

within the group with PN-sIgE ≥ 35 kIU/L. ROC analyses (Table S4 with selected data shown graphically in Figure 5) revealed that, for subjects with PN-sIgE ≥ 35 kIU/L, levels of PN-IgE were modestly related to attainment of SU (AUC = 0.76; $P = .0415$) whereas levels

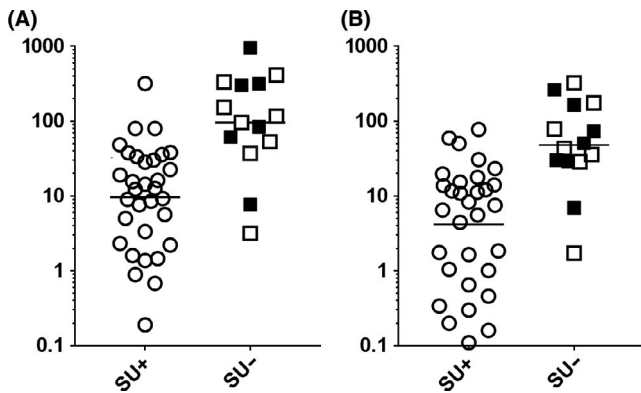


FIGURE 3 For all subjects, levels of PN-sIgE and Ara h 2-sIgE are inversely associated with attainment of sustained unresponsiveness. Data shown are for all who were dosed in the DEVIL study ($n = 46$) (A,B) (Table S1). Data from subjects who did not achieve SU due to withdrawals are shown as filled squares and those who completed OIT and did not achieve SU are shown in open squares. Cutoffs of PN-sIgE ≥ 35 kIU/L (dashed line in A) and ≥ 24 kIU/L (dashed line in B) are shown

of Ara h 2-sIgE were not significantly related to attainment of SU (AUC = 0.71; $P = .0871$). For these subjects with PN-sIgE ≥ 35 kIU/L, PN-sIgE₄ was not significantly related to attainment of SU (AUC = 0.521; $P = .877$) and the PN-sIgE/IgG₄ ratio (AUC = 0.740; $P = .076$) was less useful than PN-sIgE alone. However, the strength of IgE binding to epitope 1 (AUC = 0.79; $P = .0264$) and to epitopes 1, 5, and 6 together (AUC = 0.82; $P = .0160$) was more strongly related to attainment of SU. Using PN-IgE levels and binding to epitopes 1, 5, and 6 as variables led to the highest AUC (AUC = 0.86; $P = .0067$).

3.3 | Localization of epitopes 1, 5, and 6 of Ara h 2

Given the potential importance of IgE binding to epitopes 1, 5, and 6 of Ara h 2, we modeled the location of these epitopes onto the published structure of Ara h 2 and found that these epitopes (epitopes 1, 5 and 6) localize to contiguous areas of Ara h 2 (Figure 6). This area on Ara h 2 overlaps to a large extent with an area on Ara h 6 that was identified by modeling PN-sIgE mimotopes to surface structures.²⁷

4 | DISCUSSION

This study was designed to determine whether measurement of PN-IgE, Ara h 2-IgE, and/or IgE binding to linear epitopes of Ara h 2 and Ara h 6 has utility in the prediction of the sensitivity of peanut-allergic subjects to ingested peanut protein and/or to outcomes of OIT. Samples were diluted to contain the same amounts of peanut-specific IgE, removing the potential confounder of varied amounts of PN-sIgE. Since PN-sIgE is highly correlated with Ara h 2-sIgE ($r = .93$; $P < .0001$ in this study), normalizing on the PN-IgE data is similar to normalizing on the Ara h 2-IgE data.

There are several important findings from this analysis of data and samples from the DEVIL study.¹⁰ (a) When all subjects are analyzed together, regardless of the level of sIgE, the eliciting dose in an open challenge with peanut protein administered prior to beginning OIT was inversely associated with levels of PN-sIgE or Ara h 2-sIgE (Figure 1A). For levels of PN-sIgE, this relationship was also seen for subjects with PN-sIgE < 14 kIU/L (Figure 1B) but was lost for those subjects with PN-sIgE ≥ 14 kIU/L (Figure 1C). (b) In subjects with PN-sIgE ≥ 14 kIU/L, the eliciting dose was inversely associated with IgE binding to epitopes 5 and 6 of Ara h 2 (Figure 2) and not associated with levels of PN-sIgE or Ara h 2-sIgE (Figure 1C,F). (c) When all subjects were analyzed together, lower levels of PN-sIgE and Ara h 2-sIgE were associated with the eventual attainment of SU (Figure 3A,B). For those with PN-sIgE < 35 , the PN-sIgE data appear to be sufficient to predict outcomes from OIT, since 25 of 27 (93%) achieved SU. This was also true for Ara h 2-sIgE < 24 kIU/L where 28 of 30 (93%) achieved SU. However, for the patients with PN-sIgE ≥ 35 , PN-sIgE was not a good predictor since only 7 of 19 (37%) achieved SU (Table S1 and Figure 3A). This was also true for Ara h 2-sIgE ≥ 24 . (d) For subjects with PN-IgE ≥ 35 kIU/L, who have the highest risk of failing to achieve SU, levels of IgE binding to epitope 1 were associated with increased likelihood of not attaining SU, although there was considerable overlap (Figure 5C). In an ROC analysis, adding data from IgE binding to linear epitopes 1, 5, and 6 together improved the AUC and statistical significance (Table S4). (e) The linear epitopes identified in this study are potentially of clinical importance; epitopes 1, 5, and 6 of Ara h 2 fold together to form a patch that is homologous to an area on Ara h 6 that was identified by modeling PN-sIgE mimotopes to surface

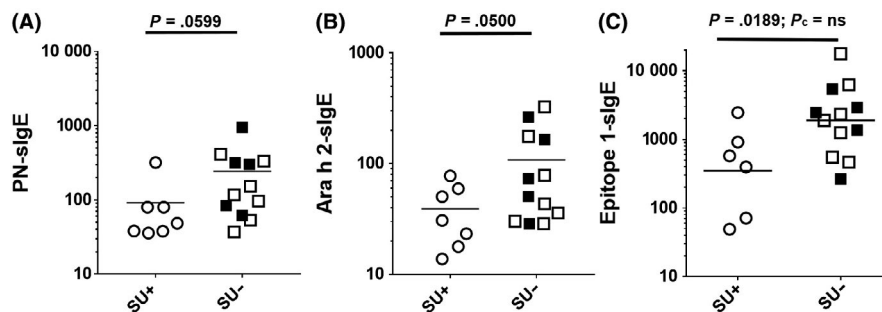


FIGURE 4 Among subjects with PN-sIgE ≥ 35 kIU/L, IgE binding to epitope 1 of Ara h 2 is inversely associated with attainment of sustained unresponsiveness. These are the samples with PN-sIgE ≥ 35 kIU/L. A, PN-sIgE; B, Ara h 2-sIgE; and C, binding of IgE to epitope 1 of Ara h 2. Data from subjects who did not achieve SU due to withdrawals are shown as filled squares and those who completed OIT and did not achieve SU are shown in open squares

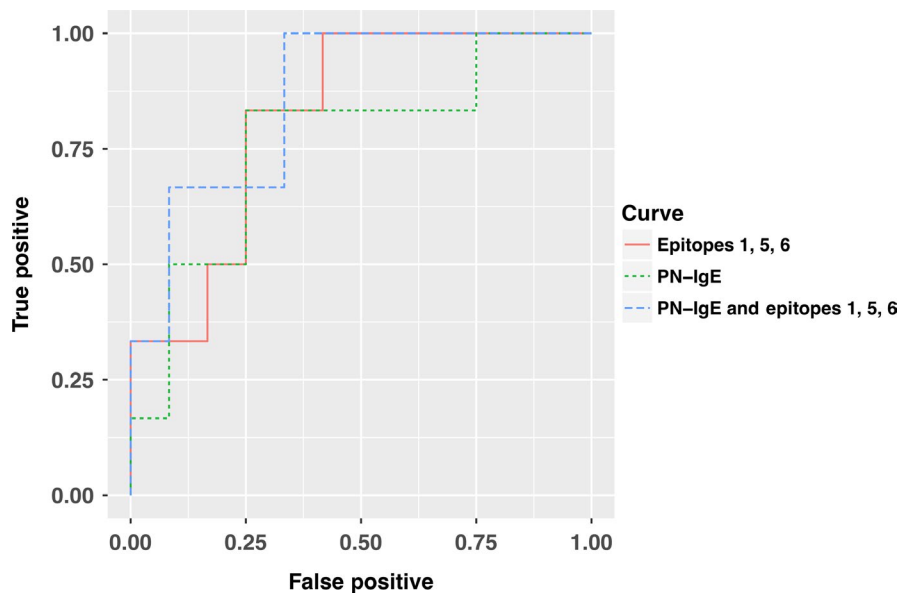


FIGURE 5 Receiving operator curve. Data are shown for subjects with PN-sIgE ≥ 35 kIU/L. An ROC curve for PN-sIgE (green dashed line); cumulative IgE binding to epitopes 1, 5 and 6 of Ara h 2 (orange solid line); and a composite analysis using PN-sIgE and cumulative IgE binding to epitopes 1, 5 and 6 of Ara h 2 (blue dashed line)

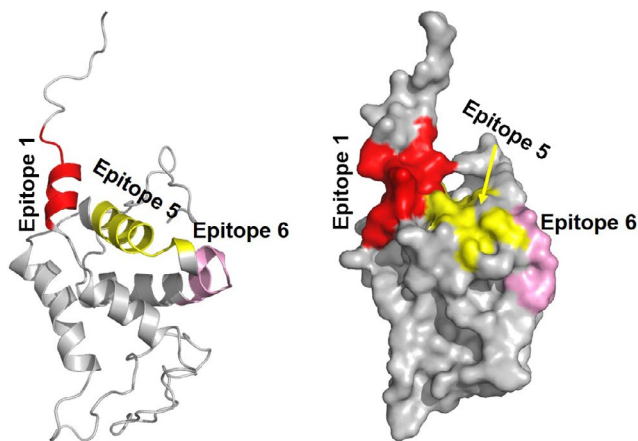


FIGURE 6 Localization of epitopes 1, 5, and 6 on the reported structure of Ara h 2. This is the X-ray crystal structure of Ara h 2 (PDB entry 3OB4) where the missing flexible loop regions were generated by modeling as described in Ref.²⁷ Ribbon structure (left) and surface analysis (right). Epitopes are color-coded: 1, red; 5, yellow; and 6, pink

structures.²⁷ Thus, this area of Ara h 2 which has now been identified in two independent analyses may be particularly important in cross-linking allergen-sIgE leading to activation of mast cells.

There are a number of important caveats. (a) The subjects studied were all preschool children, and these findings may not apply to older children or adults. (b) For IgE binding to linear epitopes, we only examined samples that had PN-IgE ≥ 14 kIU/L. The cutoff of PN-sIgE of 14 kIU/mL may be less relevant for these pediatric subjects who have been clinically challenged compared to older subjects (7-70 years).²⁴ However, since we found that, for samples with PN-sIgE < 35 kIU/L, the PN-sIgE data were as useful as the epitope data, it is unlikely that epitope data for subjects with PN-sIgE < 14 kIU/L would be informative. (c) The SU failures include dropouts and those who completed the study, making this a potentially heterogeneous group. (d) The analysis of IgE binding to epitopes of Ara h 2 that

focused on subjects with PN-sIgE ≥ 35 kIU/L was performed *post hoc*, possibly leading to inadvertent bias. (e) In the group of patients with PN-IgE > 35 , only 2 out of 6 subjects who achieved SU (SU+) had epitope-1-specific IgE levels below any of the data points for the SU- group (Figure 4C) and the remaining 4/6 are indistinguishable based on the values observed. However, another way to look at these data is that 5 of 6 subjects with PN-sIgE > 35 who achieved SU (SU+) had epitope-1-specific IgE levels below the mean of those in the SU- group. Furthermore, 11 of 12 subjects with PN-sIgE > 35 who did not achieve SU (SU-) had epitope-1-specific IgE levels above the mean of those in the SU + group (Figure 4C). Statistical analysis demonstrates these differences are significant with a $P = .0189$.

In conclusion, for the children who participated in the DEVIL study, lower levels of PN-sIgE were an excellent predictor of sensitivity to challenge at baseline and to achievement of SU. Unexpectedly, the relationships of PN- or Ara h 2-sIgE levels to sensitivity to challenge were lost in those subjects with PN-sIgE ≥ 14 kIU/L and the usefulness of measuring PN- or Ara h 2-sIgE levels for predicting the attainment of SU was lost in those subjects with PN-sIgE ≥ 35 kIU/L. In these settings, measurement of IgE binding to specific epitopes of Ara h 2 appears to have clinical value. Although these findings need to be validated in an independent cohort, it appears that, in addition to measuring PN-sIgE, measuring IgE binding to specific epitopes of Ara h 2 may be useful for stratifying patients prior to peanut challenges and prior to initiating OIT.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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TABLE E1. PN-sIgE, Ara h 2-sIgE, eliciting dose and SU status of subjects treated with OIT in the DEVIL study (shown with ascending values for PN-sIgE).

PN-specific IgE	PN-specific IgG4	PN-specific IgE/IgG4	Ara h 2-specific IgE	Eliciting dose (mg)	SU*	DEVIL ID number
0.19	0.1	2.5	0.16	300	Y	23
0.68	0.1	4.5	1.05	150	Y	6
0.89	0.0	18.2	0.34	25	Y	32
1.38	0.5	2.8	0.11	25	Y	33
1.46	0.3	4.6	0.3	15	Y	11
1.6	0.0	37.9	1.76	75	Y	7
2.21	0.1	30.9	0.2	25	Y	5
2.33	1.0	2.2	0.46	75	Y	37
3.18	0.4	8.1	1.73	75	WD	15
3.36	0.1	29.7	1.65	100	Y	22
5.03	0.3	18.1	1.85	50	Y	12
5.67	0.0	151	0.65	5	Y	29
7.62	0.7	10.5	6.5	300	Y	25
7.71	0.3	27.2	6.96	25	N	44
8.52	0.6	14.1	8.34	100	Y	39
9.03	0.1	111	5.57	15	Y	8
9.26	0.0	274	1.01	1	Y	26
12.3	2.8	4.4	7.52	15	Y	40
12.7	0.8	16.9	ND	15	Y	31
14.4	0.2	64.3	14.1	15	Y	1
15.6	0.6	27.1	11.2	15	Y	3
16.2	1.6	10.2	11.7	75	Y	28

19.1	0.5	36.6	4.46	25	Y	43
22.6	1.3	17.4	12.1	5	Y	49
28.5	0.1	264	10.9	15	Y	21
30.2	0.5	56.6	15.4	15	Y	4
33.7	0.2	184	19.8	15	Y	20
35.8	0.3	137	30.7	5	Y	27
37.1	0.8	47.8	30.1	5	WD	41
37.9	0.4	86.7	13.8	5	Y	14
38.2	1.7	22.5	23.3	15	Y	24
48.6	0.3	155	17.8	50	Y	2
53.3	0.2	285	28.7	1	WD	16
61.5	5.3	11.5	28.7	25	N	30
79.8	0.3	271	50.3	50	Y	34
79.9	0.5	156	59.4	1	Y	42
84.2	1.2	70.3	50.6	75	N	35
96	2.8	34.7	43.4	5	WD	9
117	0.1	1147	35.8	15	WD	45
153	0.2	680	78.5	15	WD	36
303	0.5	572	165	15	N	47
316	1.7	188	73.5	1	N	46
319	7.6	41.8	77.5	25	Y	13
334	0.3	1021	176	15	WD	38
414	0.8	531	325	25	WD	48
952	2.5	387	264	1	N	10

*Y, achieved SU

WD, withdrew (did not complete OIT)

N, completed OIT but did not achieve SU

TABLE E2. PN-sIgE, Ara h 2-sIgE, IgE binding to relevant epitopes of Ara h 2, eliciting dose and SU status of the samples studied with microarrays. Samples shown with ascending values for PN-sIgE.

PN-specific IgE	Ara h 2-specific IgE	Net signal epitope 1	Mean z score epitope 1	Net signal epitope 5	Mean z score epitope 5	Net signal epitope 6	Mean z score epitope 6	Net signal epitopes 5+6	Eliciting dose (mg)	SU*	Devil ID number
19	4.46	290	0.41	169	-0.21	162	-0.31	331	25	Y	43
23	12.1	465	3.19	994	4.72	642	4.47	1,636	5	Y	49
28	10.9	85	-0.03	192	0.19	78	-0.04	270	15	Y	21
30	15.4	5,093	14.06	222	0.91	201	0.47	423	15	Y	4
34	19.8	206	1.46	84	-0.36	53	-0.53	137	15	Y	20
36	30.7	910	4.46	185	1.26	94	0.37	4.46	5	Y	27
37	30.1	1,243	3.56	123	-0.41	153	-0.47	276	6	WD	41
38	13.8	264	0.61	395	1.44	281	0.68	676	5	Y	14
49	17.8	71	-1.51	157	-0.29	124	-0.83	281	50	Y	2
53	28.7	1,879	8.12	458	2.11	3525	10.12	3,983	1	WD	16
62	28.7	262	0.58	74	-0.86	91	-1.43	0.58	25	N	30
80	50.3	2,449	10.73	145	0.91	129	0.59	274	50	Y	34
80	59.4	49	-1.01	190	0.27	67	-0.86	257	1	Y	42
84	50.6	2,436	5.85	214	1.05	179	0.66	393	75	N	35
96	43.4	2,314	8.75	15,784	12.64	8238	13.78	24,022	5	WD	9
117	35.8	546	4.39	27	-1.35	58	-1.13	85	15	WD	45
153	78.5	462	3.05	111	-0.84	135	-1.02	246	15	WD	36
303	165	2,875	8.5	0	-1.05	3	-0.93	3	15	N	47
316	73.5	1,364	5.61	699	3.1	63	-0.33	762	1	N	46
319	77.5	574	5.15	61	-0.75	54	-0.7	115	25	Y	13
334	176	6,208	13.83	138	-0.81	2934	-0.82	3,072	15	WD	38
414	325	17,640	22.79	153	1.41	44	-0.34	197	25	WD	48
952	264	5408	0.39	5408	-1.36	1035	3.96	1,076	1	N	10

***Y, achieved SU; WD, withdrew (did not complete OIT); N, completed OIT but did not achieve SU**

TABLE E3. Analysis of cut-off values in relationship to achieving SU

Analysis	Population	Cut off	See Figure	Specificity	Sensitivity	PPV	NPV
PN-sIgE	All participants*	35 kIU/L	2A	0.87	0.78	0.93	0.65
Ara h 2-sIgE	All participants[†]	24 kIU/L	2B	0.86	0.87	0.93	0.75

***Table E1**

[†]Table E1 except Ara h 2-sIgE datum was not available for subject #31

Table E4. ROC analyses with data from subjects with PN-sIgE>35kIU/L.

ROC analysis (n=18)	AUC	p value*
PN-IgE	0.76	0.04153
PN-IgG₄	0.52	0.8125
PN-IgE/PN-IgG₄	0.74	0.0760
Ara h 2-IgE	0.71	0.08711
Epitope 1	0.79	0.02645
Epitope 5 and 6	0.56	0.37513
Epitopes 1, 5 & 6	0.82	0.01600
Epitopes 1, 5 & 6 plus PN-IgE	0.86	0.00673

*For these analyses, a one sided t-test was used.