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Early epitope-specific IgE antibodies are predictive of childhood peanut allergy

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Abstract

Background: Peanut allergy is characterized by the development of IgE against peanut antigen.

Objective: Evaluate the evolution of epitope-specific (es)IgE and esIgG₄ in a prospective cohort of high-risk infants to determine if antibody profiles can predict peanut allergy after 4 years of age.

Methods: The endpoint was allergy status at 4⁺ years; samples from 293 children were collected at 3–15 months, 2–3 and 4⁺ years of age. Levels of specific (s)IgE and sIgG₄ to peanut and component proteins, and 50 esIgE and esIgG₄ were quantified. Changes were analyzed with mixed-effect models. Machine learning algorithms were developed to identify a combination of antigen- and epitope-specific antibodies that using 3–15-month or 2–3-year samples can predict allergy status at 4⁺ years.

Results: At 4⁺ years, 38% of children were Tolerant or 14% had Possible, 8% Convincing, 24% Serologic, and 16% Confirmed allergy. At 3–15 months esIgE profiles were similar among groups, while marked increases were evident at 2 and 4⁺ years only in Confirmed and Serologic groups. In

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Author Contributions

H.S., S.S., R.W., T.P., S.J., D.L., W.B., and R.L. designed the study and recruited patients. B.G. provided technical information and advice on the components of the Bead-Based Epitope Assay. Data was analyzed by M.S., M.SF, A.H. and P.D.; and M.S., M.SF, and H.S. drafted the paper. All authors reviewed and approved the manuscript.

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contrast, Peanut-sIgE was significantly lower in Tolerant at 3–15 month, increased in Confirmed and Serologic but decreased in Convincing and Possibly Allergic over time. An algorithm combining esIgEs with peanut-sIgE outperformed different clinically relevant IgE cut-offs, predicting allergy status on an “unseen” set of patients with AUCs of 0.84 at 3–15 months and 0.87 at 2–3-years.

Conclusions: Early epitope- plus peanut-sIgE is predictive of allergy status at 4⁺ years of age.

Capsule summary:

Profiling of epitope- and peanut-specific IgE as early as 3–15 month and 2–3 years of age can predict peanut allergy status at age 4⁺.

Keywords

peanut allergy; epitopes; antibodies; IgE; IgG₄; Ara h 1; Ara h 2; Ara h 3; Bead-Based Epitope Assay; BBEA; machine learning; precision medicine

INTRODUCTION

Peanut allergy, affecting ~2% of American children (1–3), is the most common single cause of fatal food-induced anaphylactic reactions in the United States (4, 5). Allergic reactions occur when IgE specific to peanut antigens induces degranulation of mast cells and basophils by binding to its high-affinity FcεRI receptors. On the other hand, antigen-specific IgG, specifically the IgG₄ isotype, may prevent these effector responses by cross-linking FcγRIIb receptors or out-competing IgE for allergen binding (6–12).

Peanut antigen has been widely studied over the years to understand its allergenic determinants. Sixteen immunodominant peanut proteins (13) have been identified and designated by their allergen names Ara h 1 – Ara h 17 (14, 15). Induction of IgE, i.e. allergic sensitization, to these allergens generally differs by geography, with Ara h 1 – Ara h 3 and Ara h 6 being associated with more severe allergic reactions in the United States, Australia and some European countries (16, 17).

Sicherer et al. (18) identified several factors, including greater serum levels of IgE specific to peanut and Ara h 2, associated with the development of peanut allergy in a high-risk pediatric population. However, IgE and IgG₄ are highly polymorphic, resulting in diverse patterns of allergen recognition among individuals. Several studies have shown that different regions on allergenic proteins, i.e. epitopes, are preferentially bound by IgE or IgG₄ and contribute to the symptom severity (19–28). Knowing how the antibody repertoire evolves from infancy could help identify children who are more likely to develop allergy. In this study we have evaluated the development of epitope-specific (es)IgE and esIgG₄ in a large prospective pediatric cohort to determine if early antibody profiles can predict the development of peanut allergy after 4 years of age.

METHODS

Study Participants, Allergy Status, and Serologic Measures

The study cohort consisted of 293 children at high-risk of developing peanut allergy who were originally recruited for the CoFAR2 (Consortium for Food Allergy Research) prospective observational study (18). The characteristics of the whole CoFAR2 cohort (n=511) and a detailed study design have been published previously (29, 30). Infants fulfilled at least one of the following criteria: 1) convincing allergic reaction to milk and/or egg with a positive skin prick test (SPT) to the trigger food(s), and/or 2) moderate to severe atopic dermatitis and a positive milk or egg SPT. Clinical and immunologic measures were evaluated yearly. For this sub-study, patients with sufficient samples at baseline (ages 3–15 months) and after 4 years were included; samples at 2-year visit (ages 2–3 years) were analyzed, when available.

The endpoint of this study was peanut allergy status at 4⁺ years, which consisted of several categories (Table 1) (18): Confirmed, Convincing, Serologic, Possible Allergy, and Tolerant. “Confirmed” status was determined by either a) a positive Oral Food Challenge and peanut specific (PN-s)IgE ≥ 0.35 kU_A/L or SPT ≥ 3 mm; or b) a convincing history of a clinical reaction and PN-sIgE ≥ 14 kU_A/L. “Convincing” status was based on a history suggestive of a clinical reaction and PN-sIgE between 0.35 to 14 kU_A/L and/or SPT ≥ 3 mm. A “Serologic” was defined as PN-sIgE ≥ 14 kU_A/L, but no known exposure to peanut. A “Possible Allergy” included patients with a suggestive history but PN-sIgE < 0.35 kU_A/L and SPT < 3 mm. “Tolerant” subjects were food tolerant and either had no evidence of PN-sIgE or had ingested peanut but had PN-sIgE ≤ 0.35 kU_A/L or SPT ≤ 3 mm. A convincing reaction was considered when at least 1 of the following symptoms was present: a) hives or angioedema; b) trouble breathing, wheezing, or throat tightness; c) vomiting within 1 hour of ingestion. The concentration of total IgE (kU/L), PN-sIgG₄ (ng/mL), and sIgE (kU_A/L) to peanut, Ara h 1, Ara h 2 and Ara h 3 were measured from plasma using the ImmunoCAP® system (ThermoFisher, Uppsala, Sweden). The study was approved by the local Institutional Review Boards. All the study subjects provided informed consent.

Peanut Epitope-Specific IgE and IgG₄

The peanut epitope library consisted of 50 15-mer peptides (CS Bio, CA, USA) from Ara h 1 (n=27), Ara h 2 (n=13), and Ara h 3 (n=10) allergens (Table E1). The Bead-Based Epitope Assay was carried-out as described previously (31). In brief, a master mix of avidin bead-coupled peptides (Luminex Corporation, USA) was prepared in 1xPBS+0.02% Tween-20+0.1% BSA buffer, and 100 μ L/well was added to 96-well filter plates with 3 wells containing only buffer for background estimation. Plates were incubated for 2 hours with 100 μ L of plasma (1:10 dilution), in triplicates, using the sample randomization scheme generated with *PlateDesigner* (32). Plates were washed and incubated for 30 min with 50 μ L/well of mouse anti-human antibody (2 μ g/mL IgE-PE, Cat. MA1–10375, ThermoFisher Scientific (Waltham, USA) or 0.25 μ g/mL IgG₄-PE, Cat. 9200–09, Southern Biotech (Birmingham, USA)). The beads were resuspended in 100 μ L/well of buffer and read on the Luminex200 (Luminex Corporation, USA) as Median Fluorescence Intensity (MFI). For each peptide and sample, the MFIs were log₂ normalized and the background signal was

subtracted (nMFI = normalized mean fluorescence intensity); then the plate effect was removed using linear modeling, as described in a separate publication (31).

Statistical Analysis

Statistical analyses were performed in R v3.5. Measurements of peanut-, Ara h 1-, Ara h 2-, and Ara h 3-sIgE and IgG₄ were log₁₀ transformed prior to analysis. For each sample, the overall antibody levels to all 50 epitopes were also summarized as z-scores. Group comparisons were presented as ANOVA p-values obtained by fitting a simple linear regression; to account for different age range at the 4⁺ year visit, the regression model included age at 4-year specimen collection as a covariate.

Changes in specific IgE and IgG₄ over time by status were assessed using a random intercept linear mixed-effect model with a compound-symmetric correlation structure. For the epitope-specific antibody models, the estimations were done using an empirical Bayes approach in *limma* framework to allow estimation of the variance parameters acquiring information across all epitopes (33, 34). The results are presented as either fold-changes (FCH) from 3–15 months or estimated marginal means (EMmean). P-values were adjusted for multiple testing with Benjamini-Hochberg approach, controlling the False Discovery Rate (FDR).

Machine Learning

The schematic of the machine learning pipeline is demonstrated in Figure E1. For the purpose of this modeling, patients with “Convincing” or “Possible Allergy” status were excluded. Samples were split (35) into training and validation (n=139 and 46, 80%) and final test (n=45, 20%) sets. The testing set was then set aside by giving the data to a statistician, who was not part of the study, until the final prediction algorithm was “locked”. The binary outcome was defined as either Allergic (Confirmed or Serologic) or Tolerant status at 4⁺ years.

Training of the Random Forest (RF) algorithm was performed in the following steps using functions from the *caret* package (36): 1) 300 bootstrap resamples, i.e. cross-validation sets, were created from the training data; 2) for each resample, a RF algorithm was fitted, and tuning parameter *mtry* was estimated through a 10-fold cross-validation, maximizing the area under the curve (AUC); 3) an importance metric (mean decrease in Gini index) for each feature was recorded; 4) feature was then marked “important” if its importance metric was above the median of all features; 5) the proportion of times the feature was marked “important” was recorded, i.e. bagging frequency; 6) another random forest algorithm was then fitted using only the features selected by the majority (60–100%) of the models; a model with highest AUC across bootstrapped resamples; and 7) evaluated on the unseen test set.

Different sets of features were explored, including esIgE and esIgG₄ alone or in combination with sIgE to peanut, Ara h 1, Ara h 2, and Ara h 3. Receiver operating characteristic (ROC) curves were compared with the DeLong test (37). Algorithms were run separately for features collected at 3–15 months and 2 years. Different ways of combining timepoints were

also tested: incorporating 3–15 months and 2-year data together, or the difference from 2 years to 3–15 months, or a linear discriminant projection (38).

Various sIgE cut-offs were applied to training, validation, and testing sets to benchmark machine learning algorithms against current relevant clinical definitions for peanut allergy: a) PN-sIgE greater than 0.1, 0.35, and 14 kU_A/L; b) Ara h 2-sIgE greater than 1.32 and 2 kU_A/L; c) sIgE to PN plus Ara h 1 and Ara h 2 and Ara h 3 greater than 0.35 kU_A/L.

RESULTS

Study Population

Two hundred and ninety-three children from the CoFAR2 observational cohort were included in this study (Table 1). Based on the peanut allergy status at 4⁺ years, subjects were classified into five groups: Tolerant (n=111, 38%), Possible Allergy (n=41, 14%), Convincing (n=22, 8%), Serologic (n=71, 24%), and Confirmed (n=48, 16%). At the 4⁺-year timepoint, patients' average age was 6.7±1.3 years, 70% were male, 94% Non-Hispanic, and predominantly (74%) of White/Caucasian race (Table 2). The proportion of patients with atopic diseases was high, with 69% having allergic rhinitis, 49% asthma, or 73% eczema.

Compared to the Convincing, Possible Allergy and Tolerant children, Serologic and Confirmed groups had higher levels of total IgE, and specific IgE to peanut, Ara h 1, Ara h 2, and Ara h 3 proteins (all p<0.001), with no significant differences in PN-sIgG₄ (p=0.226). Similar patterns were observed in the z-scores of 50 esIgE (p<0.001) and esIgG₄ (p=0.218).

Baseline sIgE but not esIgE is different among allergic and Tolerant patients

Early clinical and serological measures potentially characteristic of a peanut allergy diagnosis at 4⁺ years were compared at the baseline visit, at 0.8±0.3 years of age (Table E2). Consistent with the Sicherer et al. report (18), there were no differences based on the history of egg allergy or atopic diseases.

While all groups had detectable esIgE and esIgG₄ levels at baseline (Figure 1AB), their values were not significantly different from Tolerant patients. Children with Convincing peanut allergy had higher but non-significant mean esIgG₄ levels, while esIgE was greatest in the Serologic group. Additionally, IgE and IgG₄ preferentially bound neighboring, but mostly distinct epitopes: highest level of esIgG₄ (defined as 2 SDs above the overall mean) was detected for the epitopes of Ara h 1 #015, 022, 029, 173, 179, 184, and 186; while IgE mostly recognized Ara h 1 #035, 041, 184, 186, 197, and Ara h 2 #008, 019, and 021 (Figure 1B). While esIgG₄ antibodies were detected against epitopes of Ara h 1, esIgE bound epitopes on both Ara h 1 and Ara h 2 proteins. Overall, there was no correlation between the z-scores of esIgE and esIgG₄ in any of the groups (Figure E2A); however, when considering individual epitopes, esIgE and esIgG₄ to Ara h 1.186 were positively associated in all the groups (rho 0.24–0.59, all p<0.05, Figure E2B).

Conversely, PN-sIgE, Ara h 1- and Ara h 3-sIgE, and PN-sIgG₄ were significantly greater at baseline in all groups compared to the Tolerant children (Figure 1C). Ara h 2-sIgE was higher compared to the Tolerant in Confirmed, Serologic and Convincing, but not the

Possible Allergy group. The Serologic group had highest sIgE to peanut and its component proteins.

esIgE expansion occurs only in Serologic and Confirmed groups at 2 and 4⁺ years

Natural development of IgE and IgG₄ antibodies to peanut proteins and epitopes was evaluated by comparing the changes from 3–15 months to 2 and 4⁺ years of age. nMFI levels of esIgE increased only in Serologic and Confirmed groups at both time points. Compared to Tolerant, the Serologic group had higher levels of 16 esIgEs at 2 years and 47 at 4⁺ years, with similar but fewer differences in the Confirmed (9 and 39 esIgEs, Figure 2A, Table E3). The development of esIgE in Convincing and Possible Allergy was indistinguishable from that of Tolerant patients (Figure 2AB). IgE-binding epitopes detected at 2 years in Confirmed and Serologic groups had differences in biochemical properties (Table E4): higher molecular weight, less stable, more potential of binding other, less hydrophobic peptides, and a higher number of trypsin (but not pepsin) cleavage sites.

sIgE to peanut and all component proteins increased only in Serologic and Confirmed groups with no sustained changes in Tolerant patients over time (Figure E3). While sIgE levels were significantly greater in Convincing and Possible Allergy compared to Tolerant children at 3–15 months (Figure 1C), they had decreased by 4⁺ years, especially in PN-, Ara h1- and Ara h3-sIgE, with a decrease in Ara h 2-sIgE observed only in the Convincing group.

On the other hand, most of the esIgG₄ levels increased in all groups, at both 2 and 4⁺ years (Figure 2), with fewer significant esIgG₄s in Convincing at 2 years. Similarly, PN-sIgG₄ increased over time in all groups (Figure E3). Changes in expansion of esIgG₄ positively correlated with expansion of esIgE only in the Serologic group from age 3–15 months to 2 years ($\rho=0.57$, $p<0.001$) and 4⁺ years ($\rho=0.49$, $p<0.001$) (Figure E4A), and were mostly observed in the epitopes of Ara h 1 (Figure E4B).

Since at the 4⁺-year visit, age was significantly different among groups (Table 2), we carried out a sensitivity analysis, with change in esIgE or esIgG₄ levels from 3–15 months to 4⁺ years as an independent variable, and group and age as predictors. The results of the modelling were almost identical to the findings described above (Figure E5).

Early esIgE repertoire combined with PN-sIgE is predictive of allergy status at 4⁺ years

Early identification of children that will develop peanut allergy can help inform treatment strategies. Serologic data at 3–15 months and 2 years was used to predict Tolerant and Allergic (Serologic and Confirmed) status at 4⁺ years.

We first applied known clinically relevant sIgE cut-offs to our cohort (Figure 3A). For the 3–15 months data, “Ara h2-sIgE > 1.32 kU_A/L” had the highest AUC of 0.70, while others ranged from 0.62 to 0.68. However, for any of the cut-offs, only Sensitivity or Specificity were above 0.5 (Table E5); e.g., “Ara h2-sIgE > 1.32 kU_A/L” had 0.95 Specificity and only 0.42 Sensitivity. Applying clinical cut-offs to 2-year data improved the AUCs (range 0.66–0.78, all p -values<0.05, Figure 3A and S6A), with “Ara h2-sIgE > 1.32 kU_A/L” still having the best performance.

A machine learning pipeline (Figure E1) to identify the best combination of IgE- and IgG₄-binding epitopes was developed. Since we have observed that infants had sIgE to peanut and its component proteins (PNCPs, Figure 1C), combinations with those predictors were also considered. While all models had perfect performance in the training data (Figure 3A) as well as high AUC across the cross-validation re-samples (Figure E6B), a more realistic prediction accuracy was evaluated on a validation set of plasma samples (Figure 3AB, Table E5). Models using esIgE profiles alone were able to predict allergy status at 4⁺ years using 3–15 month and 2-year data with AUCs of 0.74 and 0.79.

When combined with PN-sIgE or PNCP-sIgE the prediction performance improved: adding PN-sIgE increased the AUC to 0.78 at 3–15 months and 0.90 at 2 years (Figure 3B). As with the clinical cut-off models, machine learning algorithms performed better using the 2-year samples (Figure 3C). Combining 3–15-month and 2-year data did not improve the performance of the 2-year predictions alone (Table E5). The AUCs of esIgE plus PN-sIgE or PN component-sIgE were not significantly different ($p=0.818$ at 3–15 months and $p=0.317$ at 2 years), and hence the simpler model “esIgE + PN-sIgE” was chosen for final testing. On previously “unseen” data, the AUCs were 0.84 and 0.87 at 3–15 months and 2 years, with a 2-year model misclassifying only 1/22 Tolerant and 4/23 Allergic patients (Figure 3DE).

Only a select subset of epitopes was consistently chosen, i.e. higher bagging frequency, across timepoints and model types (Figure 3F). esIgE to Ara h 2 epitopes had higher importance and were consistently selected at both baseline and 2 years. When combined with PN-sIgE or PNCP-sIgE, generally fewer IgE-binding epitopes were selected. For example, at 3–15 months, 18 epitopes were chosen in the “esIgE” model, 4 in “esIgE + PN-sIgE”, and 16 in “esIgE + PNCP-sIgE” (Table E5).

Predicting allergy in Convincing and Possibly Allergic children

The final “esIgE + PN-sIgE” model used 2-year data to predict allergy status of patients deemed “Convincing” or “Possibly Allergic” at their 4⁺-year visit. Sixty-five percent of Convincing and 55% of Possibly Allergic were classified as allergic. Overall, this predicted allergic group had higher PN-, Ara h 1 and h 2-sIgE and PN-sIgG₄, with no other differences in demographics (Table E6). Interestingly, while z-score of all 50 IgE-binding epitopes was not significant among groups ($p=0.111$), the z-score of 13 epitopes selected for the final algorithm was also higher in patients predicted to be allergic ($p=0.024$).

DISCUSSION

In this study we evaluated how peanut epitope-specific IgE and IgG₄ repertoires evolve in high-risk children enrolled in the CoFAR2 natural history study (18, 29). At 4⁺ years, both Serologic and Confirmed patients had the highest levels of sIgE to peanut, component proteins and epitopes. At the baseline visit (3–15 months of age), Confirmed, Serologic, Convincing and Possible Allergy children had similar levels of sIgE to peanut and component proteins, but only Convincing and Possible Allergy patients had significant decreases over time. This dynamic was different for the epitope-specific antibodies: while all groups looked similar at 3–15 months, only Confirmed and Serologic patients had increases in both levels and number of esIgE at 2 and then 4⁺ years; with no changes observed in

Convincing, Possible Allergy or Tolerant. Of note, the percentage of Convincing patients was about the percentage that is anticipated to outgrow their peanut allergy, i.e. ~20%; and while these children were felt to have experienced an early reaction to peanut, they may have outgrown it (39), and hence their esIgE profiles were indistinguishable from the Tolerant group.

Unlike esIgE, esIgG₄ levels increased in all patients at both 2 and 4⁺ years, with almost all epitopes recognized by this antibody. The Convincing group had the least number of esIgG₄ by 2 years but was not significantly different from the other groups by 4⁺ years. A similar pattern was observed in PN-sIgG₄.

PN-sIgE has long been investigated as a potential biomarker of symptomatic allergy, since knowing early on who will develop allergies can guide therapeutic interventions. Over the years, different studies suggested varying cut-offs of IgE specific to peanut or Ara h 2 that can be predictive of clinical reactivity and allergy development (27, 28, 40–45). In our cohort, most of these cut-offs yielded similar results, with AUC of ~0.70 predicting peanut allergy development using 3–15-month and ~0.80 with 2-year data.

We hypothesized that IgE and IgG₄ epitope-specific repertoires add more molecular granularity and may further improve current diagnostic models. We have developed a machine learning pipeline that through several iterations of algorithms selects a best combination of the most informative esIgE and/or esIgG₄ alone or in combination with other serologic measures. Using only the 3–15 months data, a model with 18 esIgE antibodies had an AUC of 0.74. Since epitope-specific antibody levels were similar among allergic and Tolerant infants, while PN-sIgE was significantly greater in allergic children, we explored the combinations of epitope- and antigen-specific IgE. Indeed, this marginally increased the predictive ability to AUCs of 0.78 and 0.80, when PN-sIgE and PN component-sIgE were included. Predictions were further improved when using 2-year instead of 3–15 months data, with the AUC of 0.90 for the esIgE plus PN-sIgE model. Similar to what we have previously observed when developing precision medicine approaches for milk immunotherapy (46), adding esIgG₄ did not provide any benefit to the predictive ability of any of the algorithms.

Several limitations of this study should be considered for evaluating epitope-based predictors: considering epitopes from other peanut allergens, having more patients with the allergy confirmed by oral food challenges, and including cohorts from broader geographic regions. We have seen an improved performance when using 2 year compared to 3–15 months samples, potentially suggesting that other intermediate timepoints, e.g., 1 and 1.5 years, should be explored. Additionally, patients could have developed allergy before the 4⁺-year timepoint, however, a lack of earlier OFCs prevents further analysis. In addition, it would be preferable to use only OFC-confirmed subjects for the development of the predictive algorithm. Unfortunately, there were only 48 subjects with OFC-confirmed allergy, which is a small number to use for modeling, especially considering that 25% of patient samples needed to be “set aside” for ‘testing’ of the algorithm. For this reason, the Serologic group was used in the model generation, even though this could introduce the risk of biasing the predictive model. However, given the peanut-specific IgE [(median & interquartile range) 90.5 kU_A/L [37.3, 163.0], Ara h 2-specific IgE [51.8 kU_A/L [16.4,

108.0], and peanut skin test wheal diameter [16.0 mm [10.5, 21.0] of these 4 – 5 y/o children, it seemed highly unlikely that they would have a negative peanut challenge and understandable why the CoFAR investigators felt it was unnecessary/unethical to perform an oral challenge in this group to confirm their allergy status.

This study demonstrates, for the first time, that machine learning algorithms combining epitope- and antigen-specific IgE levels in the first 2–3 years of life have improved accuracy in predicting peanut allergy development at 4+ years. Accurate detection of young children with persistent peanut allergy will enable clinicians to initiate appropriate therapeutic measures early when the immune system may be more amenable to sustained unresponsiveness or possibly full tolerance. In the follow up studies, we are expanding the epitope library and evaluating this algorithm on several other observational and intervention cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest:

Ms. Suprun has nothing to disclose. Dr. Sicherer reports grants and personal fees from Food Allergy Research and Education, grants from HAL Allergy, NIH-NIAID, personal fees from American Academy of Allergy, Asthma and Immunology, UpToDate, Johns Hopkins University Press, outside the submitted work; Dr. Sicherer reports grants from NIH-NIAID during the conduct of the study. Wood reports grants from NIH-NIAID, Astellas, DBV, Aimmune, Regeneron, Sanofi, other from Up To Date, outside the submitted work. Dr. Jones reports grants from NIH-NIAID, during the conduct of the study; personal fees from FARE- Food Allergy Research and Education, Aimmune Therapeutics, EMMES Corporation; grants from Aimmune Therapeutics, DBV Technologies, Astellas, Inc., FARE, NIH-NIAID, Sanofi, Regeneron, Genentech, Inc., outside the submitted work. Dr. Leung has nothing to disclose. Ms. Henning reports grants from DAIT/NIAID/NIH, during the conduct of the study. Dr. Dawson reports grants from DAIT/NIAID/NIH, during the conduct of the study. Dr. Burks reports personal fees from Aimmune Therapeutics, Inc., Astella Pharma Global Development, Consortia TX, Inc., DBV Technologies, Intrommune Therapeutics, Protta Therapeutics, N-Fold, LLC, Aravax, Hycor Biomedical, AllerGenis, kaléo, UKKO, Inc.; grants from NIH, Johns Hopkins/NIH, FARE, other from Allertein stock, Mastcell Pharmaceuticals, UpToDate royalties, outside the submitted work. In addition, Dr. Burks has patents US#7879977, US#6835824, US#6486311, US#6441142, US#5973121, US#5558869 with royalties paid. Dr. Lindblad reports grants from DAIT/NIAID/NIH, during the conduct of the study. Dr. Getts is an employee of Genisphere LLC and scientific consultant of AllerGenis LLC; in addition, Dr. Dr. Getts has a patent PCT/US15/020715 (WO) pending. Dr. Suárez-Fariñas received research funding to Mount Sinai by a grant from AllerGenis LLC. Dr. Sampson reports non-financial support from AllerGenis LLC during the conduct of the study; grants from Immune Tolerance Network; NIAID/NIH, personal fees from N-Fold Therapeutics, other from DBV Technologies, outside the submitted work; and Mount Sinai has licensed the technology for a bead-based epitope assay for food-allergen epitope analyses to AllerGenis LLC. Dr. Sampson. serves as an unpaid Board of Directors member and advisor to AllerGenis LLC.

Abbreviations:

AUC	area under the curve
BBEA	bead-based epitope assay
CoFAR	consortium of food allergy research
CI	confidence interval
EMmean	estimated marginal mean
esIg	epitope-specific immunoglobulin
FCH	fold-change
FDR	false discovery rate
OFC	oral food challenge
LDA	linear discriminant analysis
MFI	median fluorescence intensity
PN	peanut
PNCP	peanut component protein
RF	Random Forest
SD	standard deviation
SPT	skin prick test

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Clinical Implication:

If confirmed, this could enable physicians to identify infants with persistent peanut allergy for initiating early immunotherapeutic interventions.

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Figure 1:

Epitope- and antigen- specific IgE and IgG4 antibody profiles at 3–15 months by peanut allergy status. **A.** Bar-plots showing the estimated marginal mean (EMmean) of the overall z-score of 50 esIgE or IgG4 antibodies. **B.** Line-plots representing the EMmean of IgE (top) and IgG4 (bottom) directed at individual epitopes, listed on the x-axis and ordered by the peptide's position on the Ara h 1, Ara h 2 or Ara h 3 proteins. Epitopes with EMmean above 2 SDs of the overall EMmean are marked with a "plus" (+) symbol. **C.** Bar-plots showing the EMmean of total IgE, IgE specific to peanut, Ara h 1, Ara h 2, Ara h 3 proteins, and peanut-specific IgG4, modeled on the log₁₀ scale. Blue stars on top of the error bars represent significant difference from the Tolerant group (*p<0.05, **p<0.01, ***p<0.001).

Figure 2. Changes in esIgE and esIgG₄ by peanut allergy status at 4⁺ years. **A.** Log₂ fold-change (FCH) in nMFI of esIgE (right) and esIgG₄ (left) from 3–15 months to 2 or 4+ years by individual antibody-binding epitopes. Stars represent significant difference (FDR < 0.05) from the Tolerant group at 2 or 4⁺ years; and significant changes from 3–15 months are colored in red if FCH > 1.5 and FDR < 0.05 or pink if p-value < 0.05. **B.** Z-scores summarizing all 50 esIgE or esIgG₄s for each patient (points) overplayed with a group's mean and SD. Stars on top of the error bars represent significant changes over time, and on the bottom – difference from the Tolerant group (*p<0.05, **p<0.01, ***p<0.001).

Figure 3.

Machine learning based prediction of peanut allergy status at 4⁺ using 3–15 month or 2-year antibody levels. **A.** AUC of different algorithms applied to the training and validation data sets using only 3–15 months (light blue) or 2-year (dark blue) samples. **B.** ROC curves of selected models. **C.** Model comparisons based on the ROC curves and compared using the DeLong test; both colors and numbers represent p-values. **D.** ROC of the final algorithm applied to the “unseen” test data. **E.** Estimation of probability of being “allergic” (x-axis) for each patient (y-axis) in the test set; correct predictions are depicted as circles and miss-classifications as crosses. **F.** Heatmap showing selection of IgE-binding epitopes based on their bagging frequency (BF, color and number) in three different models: esIgE alone or in combination with PN-sIgE or PNCP-sIgE. Bagging frequency of 100 means that the epitope was marked “important” in 100% of models.

Table 1.

Study schematic, diagnostic criteria, and sample size.

Peanut allergy status at 4+ years	Definition	Sample Size[*]
Tolerant	Peanut tolerant	111 (38%)
Possible Allergy	Convincing history but PN-sIgE < 0.35 kU _A /L and/or SPT < 3 mm	41 (14%)
Convincing	Convincing history and PN-sIgE ≥ 0.35 kU _A /L and/or SPT ≥ 3 mm	22 (8%)
Serologic	PN-sIgE ≥ 14 kUA/L	71 (24%)
Confirmed	Positive OFC and PN-sIgE ≥ 0.35 kU _A /L or SPT ≥ 3 mm	48 (16%)

^{*} 14 participants were missing a sample at the 2–3-year time point: 2 tolerant, 1 possible allergy, 2 convincing, 4 serologic, and 5 confirmed.

Demographic and clinical characteristics of patients at 4+ years by peanut allergy status.

Table 2:

	Overall (n = 293)	Tolerant (n = 111)	Possible Allergy (n = 41)	Convincing (n = 22)	Serologic (n = 71)	Confirmed (n = 48)	p	p*
Age at a 4+-year visit, years	6.1 [5.7, 7.9]	5.9 [5.5, 6.3]	6.0 [5.7, 6.1]	7.1 [5.9, 8.2]	6.9 [5.7, 8.3]	8.1 [7.5, 8.5]	<0.001	
Age at a 2-year visit, years	2.9 [2.7, 3.0]	2.9 [2.7, 3.1]	2.9 [2.8, 3.1]	2.9 [2.7, 3.1]	2.8 [2.6, 3.0]	2.8 [2.6, 3.0]	0.129	
Male (%)	203 (69.3)	77 (69.4)	28 (68.3)	15 (68.2)	54 (76.1)	29 (60.4)	0.503	
Race (%)							0.006	
Asian	24 (8.2)	7 (6.3)	1 (2.4)	1 (4.5)	13 (18.3)	2 (4.2)		
Black/African American	43 (14.7)	15 (13.5)	7 (17.1)	3 (13.6)	14 (19.7)	4 (8.3)		
Other	8 (2.7)	1 (0.9)	4 (9.8)	1 (4.5)	1 (1.4)	1 (2.1)		
White	218 (74.4)	88 (79.3)	29 (70.7)	17 (77.3)	43 (60.6)	41 (85.4)		
Non-Hispanic/Non-Latino (%)	276 (94.2)	107 (96.4)	38 (92.7)	20 (90.9)	67 (94.4)	44 (91.7)	0.707	
Rhinitis (%)	200 (68.5)	68 (61.8)	27 (65.9)	12 (54.5)	53 (74.6)	40 (83.3)	0.032	0.179
Asthma (%)	140 (47.8)	33 (29.7)	22 (53.7)	12 (54.5)	45 (63.4)	28 (58.3)	<0.001	0.001
Eczema (%)	215 (73.4)	82 (73.9)	31 (75.6)	19 (86.4)	53 (74.6)	30 (62.5)	0.289	0.353
Egg Allergy (%)							<0.001	0.001
Confirmed	27 (9.2)	3 (2.7)	2 (4.9)	1 (4.5)	17 (23.9)	4 (8.3)		
Serologic	22 (7.5)	1 (0.9)	7 (17.1)	0 (0.0)	14 (19.7)	0 (0.0)		
Convincing	32 (10.9)	8 (7.2)	7 (17.1)	6 (27.3)	7 (9.9)	4 (8.3)		
Possible Allergy	19 (6.5)	6 (5.4)	3 (7.3)	2 (9.1)	4 (5.6)	4 (8.3)		
Tolerant	193 (65.9)	93 (83.8)	22 (53.7)	13 (59.1)	29 (40.8)	36 (75.0)		
Milk Allergy (%)							<0.001	<0.001
Confirmed	37 (12.6)	6 (5.4)	8 (19.5)	1 (4.5)	19 (26.8)	3 (6.2)		
Serologic	3 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (4.2)	0 (0.0)		
Convincing	43 (14.7)	11 (9.9)	10 (24.4)	3 (13.6)	16 (22.5)	3 (6.2)		
Possible Allergy	7 (2.4)	2 (1.8)	3 (7.3)	1 (4.5)	1 (1.4)	0 (0.0)		
Tolerant	203 (69.3)	92 (82.9)	20 (48.8)	17 (77.3)	32 (45.1)	42 (87.5)		
Other Food Allergy (%)	119 (40.6)	26 (23.4)	24 (58.5)	9 (40.9)	46 (64.8)	14 (29.2)	<0.001	<0.001
SPT – Peanut, mm	8.0 [0.0, 15.0]	0.0 [0.0, 3.1]	9.0 [5.0, 15.4]	8.2 [4.4, 12.0]	16.0 [10.5, 21.0]	12.2 [7.8, 21.1]	<0.001	<0.001

	Overall (n = 293)	Tolerant (n = 111)	Possible Allergy (n = 41)	Convincing (n = 22)	Serologic (n = 71)	Confirmed (n = 48)	p	p*
Total IgE, kU/L	333 [121, 721]	156 [57, 418]	240 [129, 474]	262 [163, 389]	749 [371, 1771]	449 [242, 927]	<0.001	<0.001
Peanut-sIgE, kU_A/L	4.6 [0.3, 47.8]	0.1 [0.0, 1.1]	3.9 [0.8, 7.3]	2.7 [0.5, 6.3]	90.5 [37.3, 163.0]	35.9 [7.1, 113.8]	<0.001	<0.001
Ara h 1-sIgE, kU_A/L	0.2 [0.0, 7.4]	0.0 [0.0, 0.1]	0.1 [0.0, 0.5]	0.0 [0.0, 0.2]	16.8 [2.6, 65.1]	6.5 [0.2, 46.0]	<0.001	<0.001
Ara h 2-sIgE, kU_A/L	1.0 [0.0, 26.5]	0.0 [0.0, 0.1]	0.2 [0.0, 2.7]	1.3 [0.4, 2.5]	51.8 [16.4, 108.0]	23.7 [3.8, 73.9]	<0.001	<0.001
Ara h 3-sIgE, kU_A/L	0.1 [0.0, 1.0]	0.0 [0.0, 0.1]	0.1 [0.0, 0.1]	0.1 [0.0, 0.1]	4.2 [0.7, 13.2]	0.5 [0.1, 10.4]	<0.001	<0.001
Peanut-sIgG₄, ng/mL	0.9 [0.3, 2.7]	0.8 [0.2, 2.9]	0.5 [0.2, 1.0]	0.4 [0.1, 1.2]	1.4 [0.8, 4.8]	0.9 [0.4, 2.7]	<0.001	0.226
esIgE z-score	2.0(7.6)	-1.7 (2.9)	-1.4 (1.7)	-2.1 (2.0)	9.0 (9.6)	5.0 (8.1)	<0.001	<0.001
esIgG₄ z-score	3.8(7.1)	3.7 (7.3)	1.6 (6.9)	5.7 (5.6)	3.5 (5.8)	5.9 (8.6)	0.057	0.218

Continuous variables are presented as a mean and standard deviation or median and 1st – 3rd quartile; categorical variables are reported as a frequency and percent. Comparisons were tested using Wilcoxon Mann-Whitney, ANOVA or a Chi-squared test. ANOVA p-values and age adjusted p-values (p*) were obtained by fitting a linear regression model.