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

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Background: Peanut allergy is characterized by the development of IgE against peanut antigen.
Objective: We sought to evaluate the evolution of epitope-specific (es)IgE and esIgG$_4$ in a prospective cohort of high-risk infants to determine whether antibody profiles can predict peanut allergy after age 4 years.
Methods: The end point was allergy status at age 4$^+$ years; samples from 293 children were collected at age 3 to 15 months and 2 to 3 and 4$^+$ years. Levels of specific (s)IgE and sIgG$_4$ to peanut and component proteins, and 50 esIgE and esIgG$_4$ were quantified. Changes were analyzed with mixed-effects models. Machine learning algorithms were developed to identify a combination of antigen- and epitope-specific antibodies that using 3- to 15-month or 2- to 3-year samples can predict allergy status at age 4$^+$ years.
Results: At age 4$^+$ years, 38% of children were Tolerant or 14% had Possible, 8% Convincing, 24% Serologic, and 16% Confirmed allergy. At age 3 to 15 months, esIgE profiles were similar among groups, whereas marked increases were evident at age 2 and 4$^+$ years only in Confirmed and Serologic groups. In contrast, peanut sIgE level was significantly lower in the Tolerant group at age 3 to 15 months, increased in Confirmed and Serologic groups but decreased in Convincing and Possibly Allergic groups over time. An algorithm combining esIgEs with peanut sIgE outperformed different clinically relevant IgE cutoffs, predicting allergy status on an "unseen" set of patients with area under the curves of 0.84 at age 3 to 15 months and 0.87 at age 2 to 3 years.
Conclusions: Early epitope-specific plus peanut-specific IgE is predictive of allergy status at age 4$^+$ years. (J Allergy Clin Immunol 2020;146:1080-8.)

Key words: *Peanut allergy, epitopes, antibodies, IgE, IgG$_4$, Ara h 1, Ara h 2, Ara h 3, Bead-Based Epitope Assay, machine learning, precision medicine*

Peanut allergy, affecting approximately 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. However, antigen-specific IgG,

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Abbreviations used
AUC: Area under the curve
CoFAR: Consortium of Food Allergy Research
es: Epitope-specific
OFC: Oral food challenge
MFI: Median fluorescence intensity
PNCP: Peanut component protein
PN-s: Peanut specific
SPT: Skin prick test

specifically the IgG_4 isotype, may prevent these effector responses by cross-linking FcγRIIb receptors or outcompeting 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 to Ara h 17.[14,15] Induction of IgE, that is, allergic sensitization, to these allergens generally differs by geography, with Ara h 1 to 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 IgG_4 are highly polymorphic, resulting in diverse patterns of allergen recognition among individuals. Several studies have shown that different regions on allergenic proteins, that is, epitopes, are preferentially bound by IgE or IgG_4 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 esIgG_4 in a large prospective pediatric cohort to determine whether early antibody profiles can predict the development of peanut allergy after age 4 years.

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 1 of the following criteria: (1) convincing allergic reaction to milk and/or egg with a positive skin prick test (SPT) result to the trigger food(s) and/or (2) moderate to severe atopic dermatitis and a positive milk or egg SPT result. Clinical and immunologic measures were evaluated yearly. For this substudy, patients with sufficient samples at baseline (ages 3-15 months) and after age 4 years were included; samples at 2-year visit (ages 2-3 years) were analyzed, when available.

The end point of this study was peanut allergy status at age 4^+ years, which consisted of several categories (Table I)[18]: Confirmed, Convincing, Serologic, Possible Allergy, and Tolerant. "Confirmed" status was determined by either (a) a positive oral food challenge (OFC) and peanut specific (PN-s)IgE greater than or equal to 0.35 kU_A/L or SPT wheal size greater than or equal to 3 mm or (b) a convincing history of a clinical reaction and PN-sIgE greater than or equal to 14 kU_A/L. "Convincing" status was based on a history suggestive of a clinical reaction and PN-sIgE between 0.35 and 14 kU_A/L and/or SPT wheal size greater than or equal to 3 mm. A "Serologic" status was defined as PN-sIgE level greater than or equal to 14 kU_A/L, but no known exposure to peanut. A "Possible Allergy" status included patients with a suggestive history but PN-sIgE level less than 0.35 kU_A/L and SPT wheal size less than 3 mm. "Tolerant" subjects were food tolerant and either had no evidence of PN-sIgE or had ingested peanut but had PN-sIgE level greater than or equal to 0.35 kU_A/L or SPT wheal size greater than or equal to 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; or (c) vomiting within 1 hour of ingestion. The concentrations of total IgE (kU/L), PN-sIgG_4 (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.

TABLE I. Study schematic, diagnostic criteria, and sample size

Peanut allergy status at age 4^+ y	Definition	Sample size, n (%)*
Tolerant	Peanut tolerant	111 (38)
Possible Allergy	Convincing history but PN-sIgE < 0.35 kU_A/L and/or SPT wheal size < 3 mm	41 (14)
Convincing	Convincing history and PN-sIgE ≥ 0.35 kU_A/L and/or SPT wheal size ≥ 3 mm	22 (8)
Serologic	PN-sIgE ≥ 14 kU_A/L	71 (24)
Confirmed	Positive OFC and PN-sIgE ≥ 0.35 kU_A/L or SPT wheal size ≥ 3 mm	48 (16)

*A total of 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.

Peanut epitope-specific IgE and IgG_4

The peanut epitope library consisted of 50 15-mer peptides (CS Bio, Menlo Park, Calif) from Ara h 1 (n = 27), Ara h 2 (n = 13), and Ara h 3 (n = 10) allergens (see Table E1 in this article's Online Repository at www.jacionline.org). The Bead-Based Epitope Assay was carried out as described previously.[31] In brief, a master mix of avidin bead-coupled peptides (Luminex Corporation, Austin, Tex) was prepared in 1× PBS + 0.02% Tween-20 + 0.1% BSA buffer, and 100 μL/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 μL of plasma (1:10 dilution), in triplicates, using the sample randomization scheme generated with *PlateDesigner*.[32] Plates were washed and incubated for 30 minutes with 50 μL/well of mouse antihuman phycoerythrin (PE) coupled antibody (2 μg/mL IgE-PE, Cat. MA1-10375, ThermoFisher Scientific, Waltham, Mass or 0.25 μg/mL of IgG_4-PE, Cat. 9200-09, Southern Biotech, Birmingham, Ala). The beads were resuspended in 100 μL/well of buffer and read on the Luminex200 (Luminex Corporation) as median fluorescence intensity (MFI). For each peptide and sample, the MFIs were $\log_2$ 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 (R Foundation for Statistical Computing, Vienna, Austria). Measurements of peanut-, Ara h 1-, Ara h 2-, and Ara h 3-sIgE and IgG_4 were $\log_{10}$ transformed before 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 IgG_4 over time by status were assessed using a random intercept linear mixed-effects 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]

TABLE II. Demographic and clinical characteristics of patients at age 4^+ y by peanut allergy status

Characteristic	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^+-y visit	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)	<.001	
Age at a 2-y visit	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)	.129	
Sex: male, n (%)	203 (69.3)	77 (69.4)	28 (68.3)	15 (68.2)	54 (76.1)	29 (60.4)	.503	
Race, n (%)							.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, n (%)	276 (94.2)	107 (96.4)	38 (92.7)	20 (90.9)	67 (94.4)	44 (91.7)	.707	
Rhinitis, n (%)	200 (68.5)	68 (61.8)	27 (65.9)	12 (54.5)	53 (74.6)	40 (83.3)	.032	.179
Asthma, n (%)	140 (47.8)	33 (29.7)	22 (53.7)	12 (54.5)	45 (63.4)	28 (58.3)	<.001	.001
Eczema, n (%)	215 (73.4)	82 (73.9)	31 (75.6)	19 (86.4)	53 (74.6)	30 (62.5)	.289	.353
Egg allergy, n (%)							<.001	.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, n (%)							<.001	<.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, n (%)	119 (40.6)	26 (23.4)	24 (58.5)	9 (40.9)	46 (64.8)	14 (29.2)	<.001	<.001
SPT to 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)	<.001	<.001
Total IgE (kU/L)	333 (121-721)	156 (57-418)	240 (129-474)	262 (163-389)	749 (371-1771)	449 (242-927)	<.001	<.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)	<.001	<.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)	<.001	<.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)	<.001	<.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)	<.001	<.001
Peanut-sIgG$_4$ (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)	<.001	.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	<.001	<.001
esIgG$_4$ 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	.057	.218

Continuous variables are presented as a mean ± SD or median and first to third quartile; categorical variables are reported as a frequency and percent. Comparisons were tested using Wilcoxon Mann-Whitney, ANOVA, or a χ^2 test. ANOVA *P* values and age-adjusted *P* values (*P**) were obtained by fitting a linear regression model.
*Age-adjusted.

The results are presented as either fold changes from 3 to 15 months or estimated marginal means. *P* values were adjusted for multiple testing with Benjamini-Hochberg approach, controlling the false-discovery rate.

Machine learning

The schematic of the machine learning pipeline is demonstrated in Fig E1 in this article's Online Repository at www.jacionline.org. 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 algorithm was performed in the following steps using functions from the *caret* package[36]: (1) 300 bootstrap resamples, that is, cross-validation sets, were created from the training data; (2) for each resample, a Random Forest 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) the 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, that is, bagging frequency; (6) another Random Forest algorithm was then fitted using only the features selected by most of the (60%-100%) models, the best model had the highest AUC across bootstrapped resamples, and (7) was evaluated on the unseen test set.

Different sets of features were explored, including esIgE and esIgG$_4$ alone or in combination with sIgE to peanut, Ara h 1, Ara h 2, and Ara h 3. Receiver-operating characteristic curves were compared with the DeLong test.[37] Algorithms were run separately for features collected at age 3 to 15 months and 2 years. Different ways of combining time points were also tested: incorporating 3- to 15-month and 2-year data together, or the difference from 2 years to 3 to 15 months, or a linear discriminant projection.[38]

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

RESULTS

Study population

Two hundred ninety-three children from the CoFAR2 observational cohort were included in this study (Table I). On the basis of peanut allergy status at 4^+ years, subjects were classified into 5 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 time point, patients' average age was 6.7 ± 1.3 years, 70% were males, 94% Non-Hispanic, and predominantly (74%) white (Table II). The proportion of patients with atopic diseases was high, with 69% having allergic rhinitis, 49% asthma, or 73% eczema.

Compared with the Convincing, Possible Allergy, and Tolerant groups, Serologic and Confirmed groups had higher levels of total IgE, and sIgE to peanut, Ara h 1, Ara h 2, and Ara h 3 proteins (all $P < .001$), with no significant differences in PN-sIgG$_4$ ($P = .226$). Similar patterns were observed in the z scores of 50 esIgE ($P < .001$) and esIgG$_4$ ($P = .218$).

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 age 4^+ years were compared at the baseline visit, at age 0.8 ± 0.3 years (see Table E2 in this article's Online Repository at www.jacionline.org). Consistent with the Sicherer et al[18] report, there were no differences based on the history of egg allergy or atopic diseases.

Although all groups had detectable esIgE and esIgG$_4$ levels at baseline (Fig 1, *A* and *B*), their values were not significantly different from those of Tolerant patients. Children with Convincing peanut allergy had higher but nonsignificant mean esIgG$_4$ levels, whereas esIgE level was greatest in the Serologic group. In addition, IgE and IgG$_4$ preferentially bound neighboring, but mostly distinct epitopes: highest level of esIgG$_4$ (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, whereas IgE mostly recognized Ara h 1 #035, 041, 184, 186, and 197 and Ara h 2 #008, 019, and 021 (Fig 1, *B*). esIgG$_4$ antibodies were detected against epitopes of Ara h 1, whereas 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 esIgG$_4$ in any of the groups (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org); however, when considering individual epitopes, esIgE and esIgG$_4$ to Ara h 1.186 were positively associated in all the groups (ρ, 0.24-0.59; all $P < .05$; Fig E2, *B*).

Conversely, PN-sIgE, Ara h 1-sIgE and Ara h 3-sIgE, and PN-sIgG$_4$ were significantly greater at baseline in all groups compared with the Tolerant children (Fig 1, *C*). Ara h 2-sIgE level was higher compared with the Tolerant group in Confirmed, Serologic, and Convincing groups, but not the Possible Allergy group. The Serologic group had the highest sIgE level to peanut and its component proteins.

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

Natural development of IgE and IgG$_4$ antibodies to peanut proteins and epitopes was evaluated by comparing the changes from 3 to 15 months with age 2 and 4^+ years. nMFI levels of esIgE increased only in Serologic and Confirmed groups at both time points. Compared with the Tolerant group, 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, Fig 2, *A*; see Table E3 in this article's Online Repository at www.jacionline.org). The development of esIgE in Convincing and Possible Allergy groups was indistinguishable from that of Tolerant group patients (Fig 2, *A* and *B*). IgE-binding epitopes detected at age 2 years in Confirmed and Serologic groups had differences in biochemical properties (see Table E4 in this article's Online Repository at www.jacionline.org): higher molecular weight, less stable, more potential of binding other proteins, less hydrophobic, 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 group patients over time (see Fig E3 in this article's Online Repository at www.jacionline.org). Although sIgE levels were significantly greater in Convincing and Possible Allergy group children compared with Tolerant children at age 3 to 15 months (Fig 1, *C*), 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.

However, most of the esIgG$_4$ levels increased in all groups, at both age 2 and 4^+ years (Fig 2), with fewer significant esIgG$_4$s in the Convincing group at age 2 years. Similarly, PN-sIgG$_4$ increased over time in all groups (Fig E3). Changes in expansion of esIgG$_4$ positively correlated with expansion of esIgE only in the Serologic group from age 3 to 15 months to 2 years (ρ, 0.57; $P < .001$) and 4^+ years (ρ, 0.49; $P < .001$) (see Fig E4, *A*, in this article's Online Repository at www.jacionline.org), and were mostly observed in the epitopes of Ara h 1 (Fig E4, *B*).

Because at the 4^+-year visit age was significantly different among groups (Table II), we carried out a sensitivity analysis, with change in esIgE or esIgG$_4$ levels from age 3 to 15 months to 4^+ years as an independent variable and group and age as predictors. The results of the modeling were almost identical to the findings described above (see Fig E5 in this article's Online Repository at www.jacionline.org).

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

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

We first applied known clinically relevant sIgE cutoffs to our cohort (Fig 3, *A*). For the 3- to 15-month data, "Ara h2-sIgE > 1.32 kU$_A$/L" had the highest AUC of 0.70, whereas others ranged from 0.62 to 0.68. However, for any of the cutoffs, only sensitivity or specificity was above 0.5 (see Table E5 in this article's Online Repository at www.jacionline.org); for example, "Ara h2-sIgE > 1.32 kU$_A$/L" had a specificity of 0.95 and a sensitivity of only 0.42. Applying clinical cutoffs to 2-year data improved the AUCs (range, 0.66-0.78; all P values <.05; Fig 3, *A*; see E6, *A*, in this article's Online Repository at www.jacionline.org), with "Ara h2-sIgE >1.32 kU$_A$/L" still having the best performance.

A machine learning pipeline (Fig E1) to identify the best combination of IgE- and IgG$_4$-binding epitopes was developed. Because we have observed that infants had sIgE to peanut and its component proteins (peanut component proteins [PNCPs], Fig 1, *C*), combinations with those predictors were also

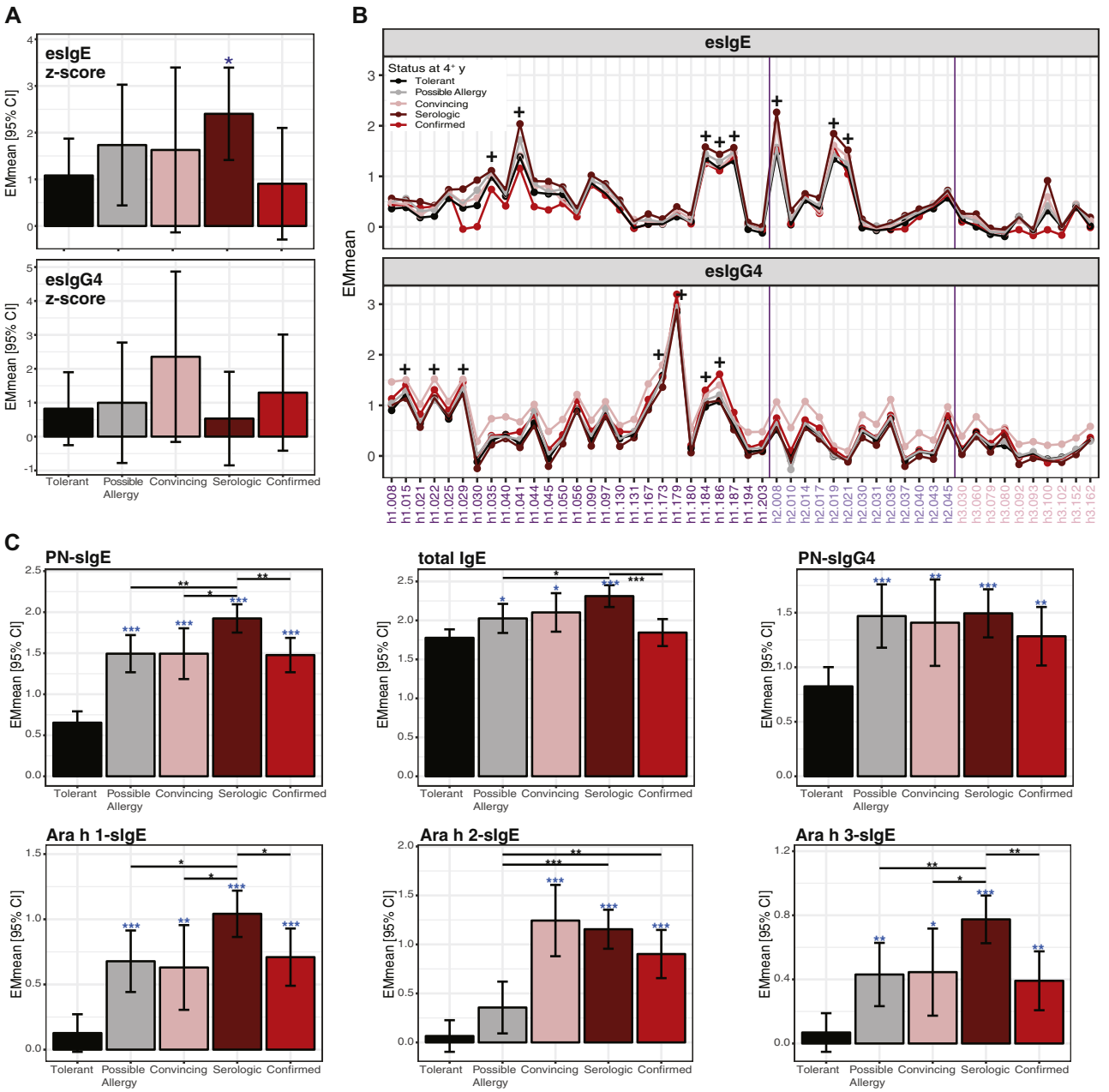

FIG 1. Epitope- and antigen-specific IgE and IgG_4 antibody profiles at age 3 to 15 months by peanut allergy status. **A,** Bar plots showing the estimated marginal mean (EMmean) of the overall *z* score of 50 esIgE or esIgG_4 antibodies. **B,** Line plots representing the EMmean of esIgE *(top)* and esIgG_4 *(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, and Ara h 3 proteins, and PN-sIgG_4, modeled on the $\log_{10}$ scale. Blue stars on top of the error bars represent significant difference from the Tolerant group (**P* < .05, ***P* < .01, ****P* < .001).

considered. Although all models had perfect performance in the training data (Fig 3, *A*) as well as high AUC across the cross-validation resamples (Fig E6, *B*), a more realistic prediction accuracy was evaluated on a validation set of plasma samples (Fig 3, *A* and *B*; Table E5). Models using esIgE profiles alone were able to predict allergy status at age 4^+ years using 3- to 15-month and 2 to 3-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 to 15 months and 0.90 at 2 years (Fig 3, *B*). As with the clinical cutoff models, machine learning algorithms performed better using the 2-year samples (Fig 3, *C*). Combining 3- to 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 PNCP-sIgE were not significantly different (P = .818 at 3-15 months and P = .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

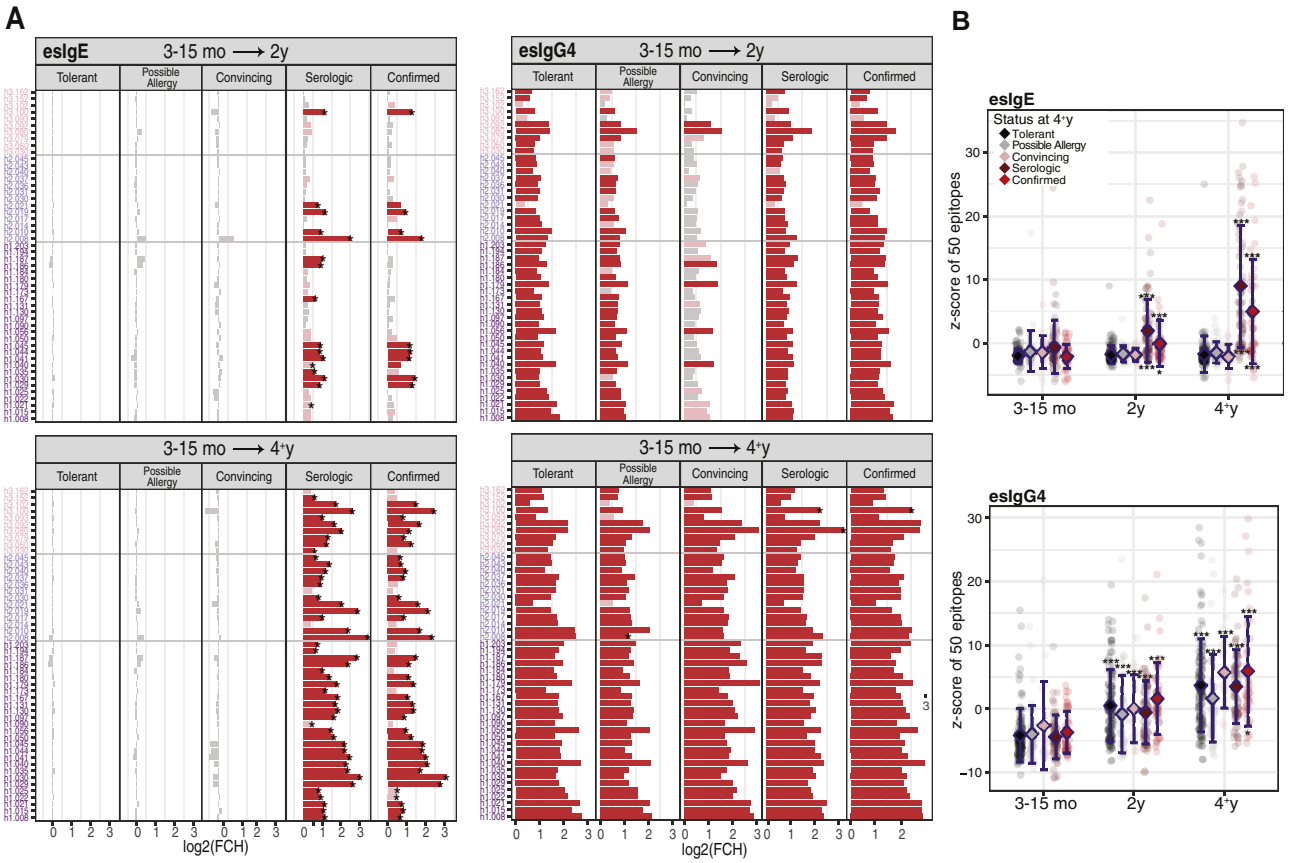

FIG 2. Changes in esIgE and esIgG$_4$ by peanut allergy status at age 4$^+$ years. **A,** Log$_2$ FCH in nMFI of esIgE *(right)* and esIgG$_4$ *(left)* from age 3 to 15 months to age 2 or 4$^+$ years by individual antibody-binding epitopes. Stars represent significant difference (FDR < 0.05) from the Tolerant group at age 2 or 4$^+$ years; significant changes from age 3 to 15 months are colored in red if FCH is more than 1.5 and FDR is less than 0.05 or pink if *P* value is less than .05. **B,** Z scores summarizing all 50 esIgE or esIgG$_4$s for each patient (points) overlayed 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* < .05, ***P* < .01, ****P* < .001). *FCH*, Fold change; *FDR*, false-discovery rate.

age 3 to 15 months and age 2 years, with a 2-year model misclassifying only 1 of 22 Tolerant and 4 of 23 Allergic patients (Fig 3, *D* and *E*).

Only a select subset of epitopes was consistently chosen, that is, higher bagging frequency, across time points and model types (Fig 3, *F*). 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 to 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 Ara h 2-sIgE and PN-sIgG$_4$, with no other differences in demographic charateristics (see Table E6 in this article's Online Repository at www.jacionline.org). Interestingly, although the *z* score of all 50 IgE-binding epitopes was not significant among groups (P = .111), the *z* score of 13 epitopes selected for the final algorithm was also higher in patients predicted to be allergic (P = .024).

DISCUSSION

In this study, we evaluated how peanut epitope-specific IgE and IgG$_4$ repertoires evolve in high-risk children enrolled in the CoFAR2 natural history study.[18,29] At age 4$^+$ years, both Serologic and Confirmed patients had the highest levels of sIgE to peanut, component proteins, and epitopes. At the baseline visit (age 3-15 months), Confirmed, Serologic, Convincing, and Possible Allergy group children had similar levels of sIgE to peanut and component proteins, but only Convincing and Possible Allergy group children had significant decreases over time. This dynamic was different for the epitope-specific antibodies: although all groups looked similar at age 3 to 15 months, only Confirmed and Serologic group children had increases in both levels and number of esIgE at age 2 to 3 years and then age 4$^+$ years, with

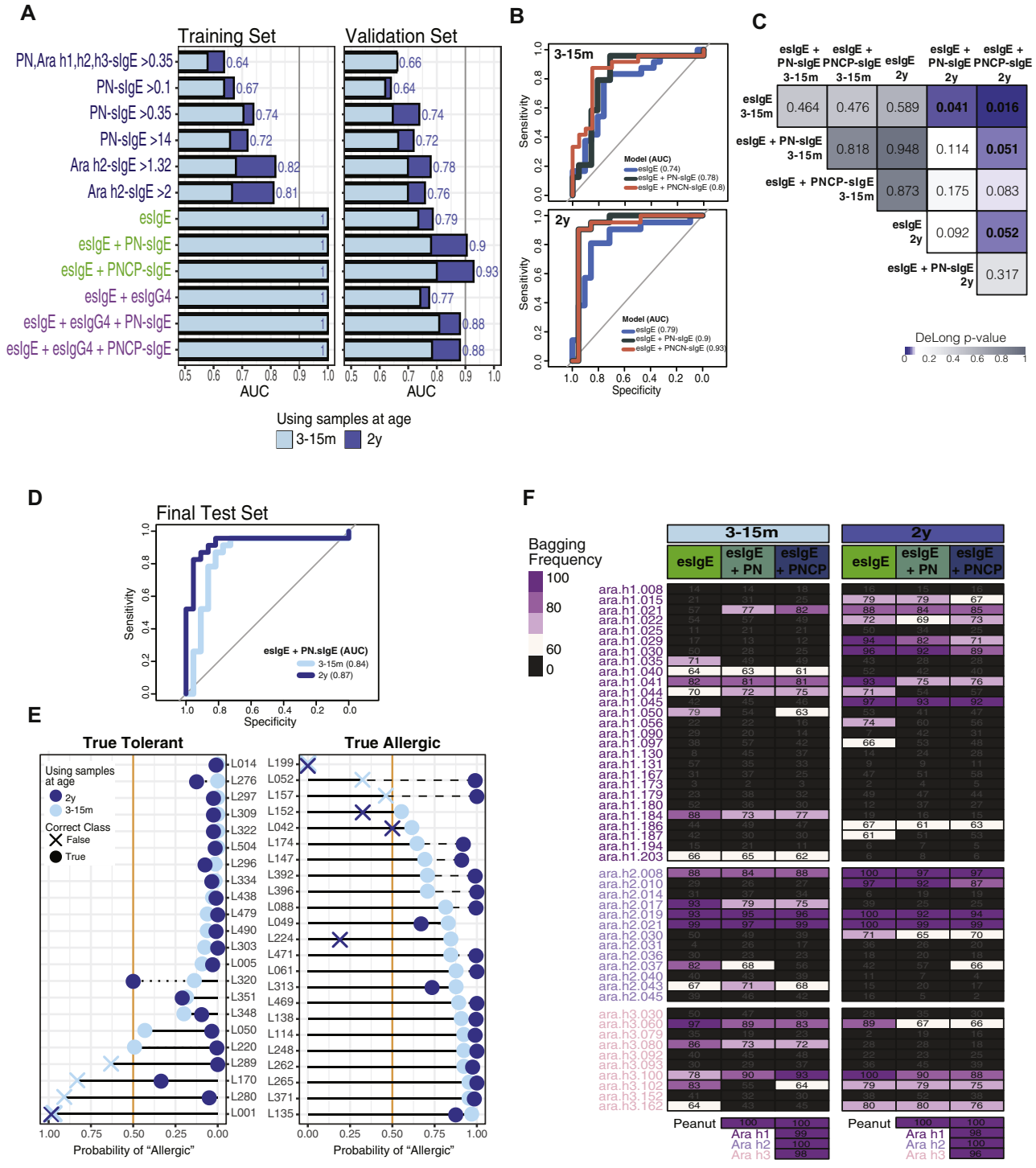

FIG 3. Machine learning–based prediction of peanut allergy status at age 4^{+} years using 3- to 15-month or 2- to 3-year antibody levels. **A,** AUC of different algorithms applied to the training and validation data sets using only 3- to 15-month *(light blue)* or 2 to 3-year *(dark blue)* samples. **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 (color and number) in 3 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. *ROC,* Receiver-operating characteristic.

no changes observed in Convincing, Possible Allergy, or Tolerant group children. Of note, the percentage of Convincing group children was about the percentage that is anticipated to outgrow their peanut allergy, that is, approximately 20%, and although 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 those of the Tolerant group children.

Unlike esIgE, esIgG$_4$ levels increased in all patients at both age 2 and 4$^+$ years, with almost all epitopes recognized by this antibody. The Convincing group children had the least number of esIgG$_4$ by age 2 years but were not significantly different from the children of other groups by age 4$^+$ years. A similar pattern was observed in PN-sIgG$_4$.

PN-sIgE has long been investigated as a potential biomarker of symptomatic allergy, because knowing early on who will develop allergies can guide therapeutic interventions. Over the years, different studies suggested varying cutoffs 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 cutoffs yielded similar results, with AUC of approximately 0.70 predicting peanut allergy development using 3- to 15-month and approximately 0.80 with 2-year data.

We hypothesized that IgE and IgG$_4$ 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 esIgG$_4$ alone or in combination with other serologic measures. Using only the 3- to 15-month data, a model with 18 esIgE antibodies had an AUC of 0.74. Because epitope-specific antibody levels were similar among Allergic and Tolerant infants, whereas 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 PNCP-sIgE were included. Predictions were further improved when using 2-year instead of 3- to 15-month 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 oral immunotherapy,[46] adding esIgG$_4$ 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 OFCs, and including cohorts from broader geographic regions. We have seen an improved performance when using 2- to 3-year compared with 3- to 15-month samples, potentially suggesting that other intermediate time points, for example, 1 and 1.5 years, should be explored. In addition, patients could have developed allergy before the 4$^+$-year time point; 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 20% 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 sIgE (median, 90.5 kU$_A$/L; interquartile range, 37.3-163.0), Ara h 2-sIgE (range, 51.8 kU$_A$/L; interquartile range, 16.4-108.0), and peanut SPT wheal diameter (range, 16.0 mm; interquartile range, 10.5-21.0) of these 4- to 5-year-old 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 to 3 years of life have improved accuracy in predicting peanut allergy development at age 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.

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

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