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Epicutaneous immunotherapy for peanut allergy modifies IgG₄ responses to major peanut allergens



To the Editor:

To date, no therapy for peanut allergy is available. Several therapeutic options are under investigation and show promise. Epicutaneous immunotherapy (EPIT) for peanut allergy has been shown to increase significantly the threshold levels of peanut-allergic patients, in particular in children.¹ The proposed mechanism of EPIT is that allergens are rapidly taken up by skin antigen-presenting cells and transported to regional lymph nodes, thereby inducing a humoral and cellular immunological response.² In a recent phase 2b clinical trial, several doses of peanut EPIT were evaluated in children, adolescents, and adults aiming to increase the patient's threshold of reactivity to peanut. The primary end point of the study was met, and the largest treatment effect was observed in children (6-11 years).¹

Using samples from the study reported by Sampson et al,¹ the current report evaluates the serological changes in children treated with either a peanut patch (250 µg peanut protein, referred to as 250-µg patch; n = 25) or a placebo patch (n = 26). Similar to the protein composition of peanut,³ the 250-µg patch contains small amounts of Ara h 6 and Ara h 2, larger amounts of Ara h 1, while Ara h 3 is the most abundant protein (see [Table E1](#) in this article's Online Repository at www.jacionline.org). The report by Sampson et al¹ showed that treatment with the 250-µg patch induced IgG₄ to total peanut protein, and in this study, we sought to investigate the specificity of the IgG₄ induction by assessing IgG₄ to peanut components following treatment.

Sera from subjects in the children treatment groups were collected at both baseline and several time points during EPIT treatment. Two testing platforms were used to analyze immunoglobulins. First, ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) was used to obtain quantitative results for peanut components Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9 (results expressed in kU_A/L for IgE and in mg_A/L for IgG₄). Ara h 6 was not available as reagent for ImmunoCAP. Second, Immuno Solid-phase Allergy Chip (ISAC; Thermo Fisher Scientific) was used because this also contains Ara h 6 in addition to the components available for

ImmunoCAP. A disadvantage of ISAC is that the results are semi-quantitative (results expressed in ISAC Standardized Units as defined by the manufacturer) and cannot be directly compared with ImmunoCAP.

Baseline sensitization of subjects to peanut components was evaluated by ImmunoCAP and ISAC. IgE determined by ISAC and ImmunoCAP correlated well for allergens available on both platforms (see [Fig E1](#) in this article's Online Repository at www.jacionline.org). [Table I](#) presents the baseline sensitization of the 51 children in the 2 groups (250-µg patch and placebo patch). No differences were observed between the 2 treatment groups ($P > .6$, Wilcoxon rank-sum test). As determined by ISAC, all subjects were sensitized to Ara h 2 and Ara h 6, with a high median IgE titer. Ara h 1 and Ara h 3 were frequently recognized but with lower IgE titers than observed for Ara h 2 or Ara h 6 ([Table I](#)). Ara h 8 and Ara h 9 were least frequently recognized with low IgE titers. IgE titers to Ara h 2 and Ara h 6 were highly correlated ([Fig 1](#); $R^2 = 0.797$), but several patients had high IgE titers to Ara h 2 while not to Ara h 6, or vice versa. Overall, the IgE titers for Ara h 6 were slightly lower than for Ara h 2. Close correlation between IgE to Ara h 2 and IgE to Ara h 6 is expected on the basis of their homology and partially shared IgE epitopes⁴ and is in line with an earlier report that used an experimental Ara h 6 ImmunoCAP showing correlation between concentrations of IgE to Ara h 2 and Ara h 6.⁵

Baseline sera and sera obtained during treatment were tested for peanut allergen-specific IgG₄ using ImmunoCAP and ISAC. [Fig E1, D](#), shows that the 2 assays correlate well for those peanut components available on both assays. [Fig 2](#) shows the induction of IgG₄ per subject per peanut component for subjects treated with the 250-µg patch. Subjects showed an increase in IgG₄ for Ara h 1, Ara h 2, Ara h 3, and Ara h 6, although the extent of this increase varied among subjects. For all subjects, Ara h 2 and Ara h 6 induced the highest levels of IgG₄. After 12 months of treatment, using ImmunoCAP, the increase in median IgG₄ for Ara h 2 was 62.7-fold, whereas Ara h 1 and Ara h 3 showed a smaller fold increase in median of 9.5- and 2.4-fold, respectively. The median IgG₄ levels for Ara h 8 and Ara h 9 did not change over time. ISAC data show that Ara h 6 induces IgG₄ to a comparable level as Ara h 2, and stronger than do the other peanut allergens ([Fig 2](#)). Ara h 2 and Ara h 6 are the strongest inducers of IgG₄ even though these allergens are present on the patch in lower amounts than Ara h 1 and Ara h 3 (see [Table E1](#)). Of note, in patients with a high IgE level for Ara h 2 and/or Ara h 6 at baseline, the induction of IgG₄ to these allergens was greater than in patients with low IgE level for Ara h 2 and/or Ara h 6 at baseline. However, the number of patients, especially those with low IgE level to Ara h 2 and/or Ara h 6, is too low to prove correlation (Pearson correlation coefficients [r] 0.61, 0.56, and 0.47, for Ara h 2 [baseline IgE by ImmunoCAP], Ara h 2 [baseline IgE by ISAC], and Ara h 6 [baseline IgE by ISAC] and corresponding P values of .0011, .0038, and .0174, respectively; data not shown).

In the placebo-treated patients, no increase in IgG₄ was observed. In this group, the baseline IgG₄ levels were comparable to those of the 250-µg patch-treated group ([Fig 2, A](#)), with medians of 0.07, 0.17, 0.15, 0.07, and 0.07 mg_A/L, respectively, for Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9. These levels did not change during the course of the study (medians at 12 months of placebo treatment for the respective peanut allergens were 0.07, 0.17, 0.14, 0.07, and 0.07 mg_A/L), in line

TABLE I. Baseline sensitization profile of study subjects randomized in the 250- μ g patch group, the placebo group, and for combined groups

Group	Peanut	Ara h 1		Ara h 2		Ara h 3		Ara h 6		Ara h 8		Ara h 9	
	CAP	CAP	ISAC	CAP	ISAC	CAP	ISAC	CAP	ISAC	CAP	ISAC	CAP	ISAC
Viaskin 250 μ g													
n positive	25 of 25	21 of 25	23 of 25	24 of 25	25 of 25	15 of 25	17 of 25	NA	25 of 25	6 of 25	9 of 25	1 of 25	0 of 25
Median	55.0	24.3	8.3	33.8	36.7	3.3	3.0	NA	25.6	0.4	0.0	0.4	0.0
LQ	20.5	0.8	1.5	11.5	12.4	0.4	0.0	NA	8.7	0.4	0.0	0.4	0.0
UQ	217.3	70.8	18.2	76.7	51.8	22.7	24.6	NA	30.6	0.4	0.7	0.4	0.0
Placebo													
n positive	26 of 26	18 of 26	21 of 26	24 of 26	26 of 26	14 of 26	19 of 26	NA	26 of 26	9 of 25	9 of 25	2 of 26	0 of 26
Median	89.8	7.7	10.3	38.8	21.7	0.8	2.3	NA	25.1	0.4	0.2	0.4	0.0
LQ	17.8	0.4	1.7	5.7	12.0	0.4	0.0	NA	7.2	0.4	0.0	0.4	0.0
UQ	284.6	57.4	30.0	94.2	56.7	21.4	24.8	NA	40.0	0.6	0.5	0.4	0.0
All													
n positive	51 of 51	39 of 51	44 of 51	48 of 51	51 of 51	29 of 51	36 of 51	NA	51 of 51	15 of 51	15 of 51	3 of 51	0 of 51
Median	55.0	11.3	9.7	34.2	29.5	1.3	3.0	NA	25.5	0.4	0.0	0.4	0.0
LQs	17.8	0.6	1.5	6.8	12.0	0.4	0.0	NA	8.5	0.4	0.0	0.4	0.0
UQ	284.6	67.3	23.4	94.2	56.7	22.7	24.8	NA	36.5	0.6	0.7	0.4	0.0

CAP, ImmunoCAP; LQ, lower quartile; NA, not available (allergen Ara h 6 is not available as ImmunoCAP reagent); Peanut, total peanut extract; UQ, upper quartile.

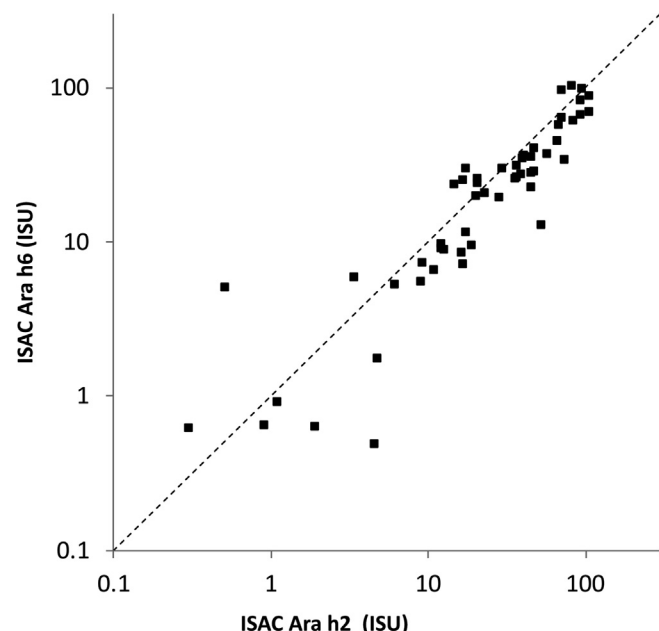


FIG 1. Correlation of IgE to Ara h 2 and IgE to Ara h 6. IgE levels to both Ara h 2 and IgE to Ara h 6 were determined with ISAC. ISU, ISAC Standardized Units.

with the noted absence of IgG₄ induction for total peanut in this group.¹ The combined results show that peanut EPIT strongly induces IgG₄ to Ara h 2 and Ara h 6, which are known as the most important peanut allergens,⁶ and to a lesser extent to Ara h 1 and Ara h 3.

Baseline sera and sera obtained during treatment were also tested for peanut allergen-specific IgE, using ImmunoCAP. Fig E2 in this article's Online Repository at www.jacionline.org shows an increase of about 2-fold during the first half year of EPIT, followed by a decrease at 1 year of EPIT treatment. This was observed for Ara h 1, Ara h 2, and Ara h 3 (Ara h 6 not available on ImmunoCAP), while for Ara h 8 and Ara h 9 the median IgE levels were low and no clear trend could be observed.

Initial increase in IgE followed by a decrease at 1 year of treatment is often seen in allergen immunotherapy, and this was also described for total peanut IgE in this current treatment population.¹

Induction of IgG₄ has been described for other investigational therapies for peanut allergy. Oral immunotherapy (OIT) treatment showed induction of IgG₄ to Ara h 1 and Ara h 2, and to a lesser extent to Ara h 3.^{7,8} The strongest response was found for Ara h 2, with an increase in median compared with baseline of about 25-fold after 1 year of treatment.⁸ A similar pattern of IgG₄ induction was found with sublingual immunotherapy (SLIT), with Ara h 2 again being the strongest IgG₄ inducer, showing an increase in median of about 10-fold compared with baseline.⁹ The OIT and SLIT studies did not evaluate IgG₄ responses to Ara h 6. The fold increases in component-specific IgG₄ achieved by EPIT shown here are comparable to those obtained with OIT⁸ and SLIT.⁹ In terms of absolute amounts of IgG₄ as determined with ImmunoCAP, we show that 12 months of peanut EPIT results in median concentrations of 0.76, 7.52, and 0.33 mg_A/L for Ara h 1, Ara h 2, and Ara h 3, respectively. These concentrations are similar, or slightly higher in the case of Ara h 2, to those found after 12 months of OIT⁸ or SLIT.⁹ These changes in IgG₄ were observed with treatment using only 250 μ g of peanut protein ($\sim$ 1/1000 of 1 peanut) applied epicutaneously daily.

In conclusion, our study demonstrates that EPIT for peanut allergy induces IgG₄ to major peanut allergens, comparable in quantity and specificity to other investigational treatment modalities for peanut allergy. Furthermore, our study shows that Ara h 6 is an important allergen in terms of sensitization and that EPIT induces a strong IgG₄ response to this allergen.

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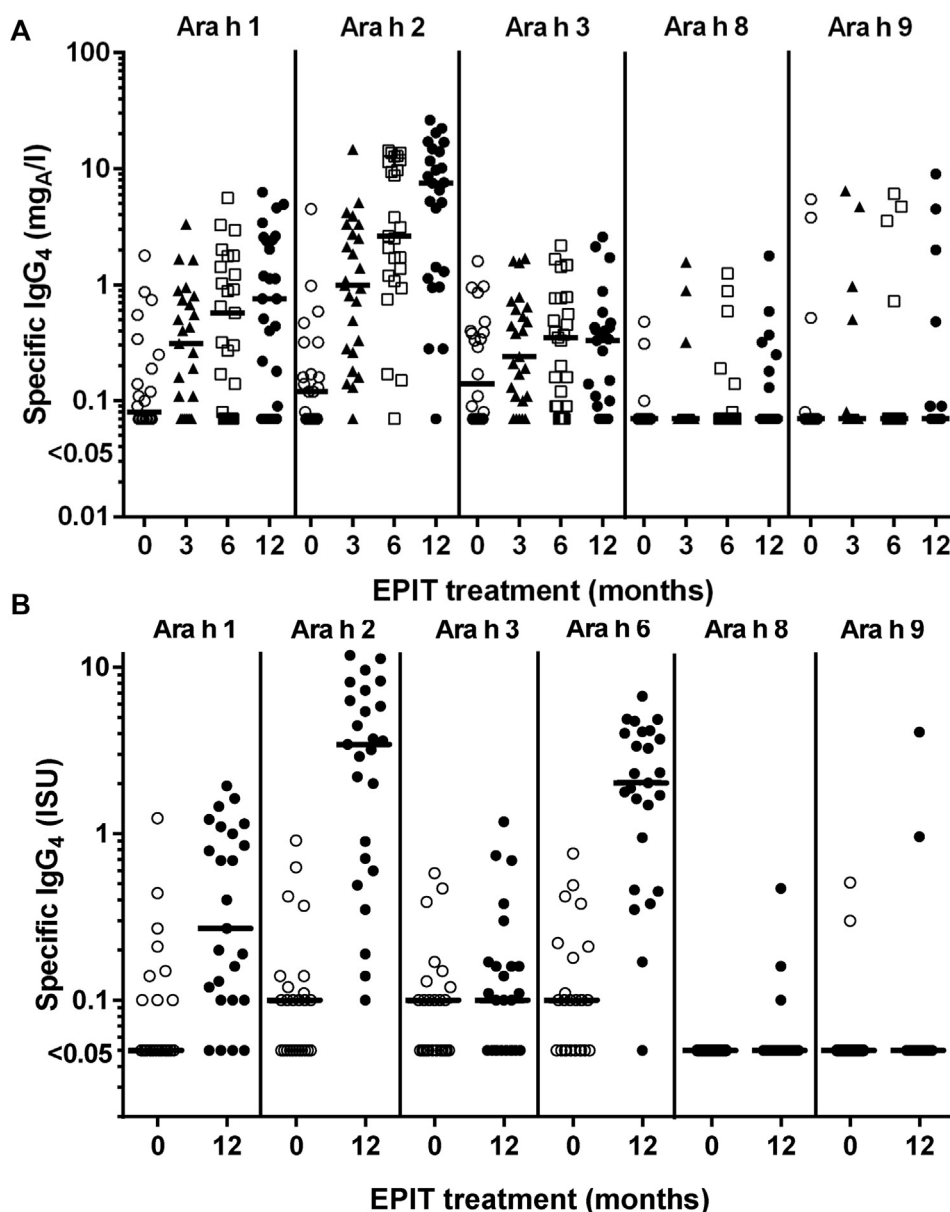


FIG 2. Induction of IgG₄ to individual peanut components by EPIT with 250-µg patch. **A**, Data obtained with ImmunoCAP. **B**, Data obtained with ISAC. Numbers on x-axis represent months of EPIT (0 is baseline). Where less than 25 markers per time point are plotted, the markers at less than 0.05 ISU for ISAC and less than 0.07 mgA/L for ImmunoCAP represent multiple markers up to a total of 25. Bars show medians. ISU, ISAC Standardized Units. Open circles, baseline; filled triangles, 3 months; open squares, 6 months; filled circles, 12 months.

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Eosinophil progenitor levels correlate with tissue pathology in pediatric eosinophilic esophagitis



To the Editor:

Eosinophilic esophagitis (EoE) is a chronic inflammatory disease of the gastrointestinal tract characterized by esophageal eosinophilia. Current EoE management comprises serial, invasive endoscopies with esophageal biopsy to diagnose and subsequently monitor treatment efficacy. A less-invasive, blood-based biomarker to monitor EoE disease activity would substantially advance EoE management; however, such a biomarker is yet to be identified.

Peak eosinophil count (PEC) of 15 intraepithelial eosinophils or more within at least 1 hpf of esophageal biopsy remains the consensus threshold for histologically diagnosing active disease. However, pathologic features other than eosinophilic inflammation are associated with active EoE. Recently, Collins et al¹ described the EoE histologic scoring system (EoEHSS), which provides a standardized method to objectively assess the severity and extent of multiple pathologic features of EoE within esophageal biopsies. The EoEHSS composite score outperformed the PEC in differentiating patients with treated and untreated EoE.¹ In addition, various EoEHSS elements were associated with distinct disease endotypes.² These data demonstrate the importance of pathologic features distinct from eosinophil infiltration in assessing EoE disease activity.

Eosinophil progenitor cells (EoPs) are eosinophil lineage-committed, CD34⁺ progenitor cells that primarily reside in the bone marrow. Growing evidence demonstrates that EoPs mobilize into the peripheral blood during allergic responses and accumulate within allergen-exposed tissues. The functional significance of mobilized EoPs remains unclear, but it has been postulated that these cells propagate local tissue eosinophilia through *in situ* proliferation and maturation into eosinophils and secretion of IL-5.³⁻⁵ Moreover, studies using isolated peripheral blood CD34⁺ hematopoietic stem/progenitor cells have demonstrated that these cells can be potent producers of T_H2 cytokines, particularly IL-13.⁶ Consequently, EoPs may be participating in propagating T_H2 inflammatory responses.

We previously demonstrated that EoP levels in the peripheral blood were significantly increased in patients with active EoE.⁷ Notably, a cutoff value of less than 15.5 absolute EoPs/mL was associated with 100% sensitivity for differentiating EoE disease

activity, suggesting that peripheral blood EoP levels may represent a novel disease-monitoring biomarker for pediatric patients with EoE.⁷ No clinical studies to date have evaluated the relationship between peripheral blood EoP levels and the pathologic changes assessed by the EoEHSS. We hypothesized that EoP levels would correlate with the histologic changes observed in the esophagus during active EoE and would therefore correlate with EoEHSS disease scores.

To test our hypothesis, biopsies from our previously published pediatric EoE cohort⁷ underwent blinded assessment by a pathologist using the EoEHSS, as previously described.¹ The EoEHSS evaluates 8 pathologic features commonly seen in EoE esophageal biopsies (see Table E1 in this article's Online Repository at www.jacionline.org). The severity (grade) and extent (stage) of these pathologic changes within the esophageal biopsies were scored and ratios generated by dividing the score obtained by the maximum possible score. The grade and stage ratios were combined to generate a total EoEHSS composite ratio. The cohort included subjects aged 1 to 18 years with an EoE diagnosis and previous failure of proton pump inhibitor therapy to abate their esophageal eosinophilia.⁷ A total of 31 patients were divided into 2 groups on the basis of their PEC: inactive EoE (PEC < 15 eosinophils/hpf; n = 14) and active EoE (PEC ≥ 15/hpf; n = 17) (see Table E2 in this article's Online Repository at www.jacionline.org). Patient age, sex, and atopic status were not significantly different between these groups. EoPs were identified within isolated PBMCs by flow cytometry as the live, CD45RA⁻, CD34^{High}+, CD38⁺, and CD125⁺ (IL-5Rα) events. Notably, EoPs were significantly increased in the peripheral blood of patients with active EoE.⁷

We previously demonstrated that EoP levels significantly correlated with the maximum severity of esophageal eosinophilia (ie, PEC), which remains the criterion standard for assessing disease activity.⁷ Severity is a principal metric, but the extent of tissue involvement is also essential to accurately assess disease activity. Indeed, both grade (severity) and stage (extent) are measures routinely used to evaluate other inflammatory gastrointestinal diseases.⁸ There is a distinct advantage to using the EoEHSS, which evaluates grade and stage, compared with the PEC alone.¹ To date, however, composite EoEHSS score thresholds have not been determined for active disease. Consequently, we determined whether EoP levels correlated with EoEHSS histologic metrics. The EoEHSS composite ratio (combined grade and stage scores) was significantly higher in active EoE than in inactive EoE ($P < .001$; Mann-Whitney test, Fig 1, A). Next, we evaluated the relationship between EoP levels and the EoEHSS composite ratio. We observed a significant, positive correlation between the absolute blood EoP levels and the EoEHSS composite ratio (Spearman $\rho = 0.53$; $P = .02$; Fig 1, B) and between EoP levels and both grade (Spearman $\rho = 0.54$; $P = .002$; Fig 1, C) and stage (Spearman $\rho = 0.50$; $P = .005$; Fig 1, D) ratio scores.

Given that the EoEHSS evaluates both eosinophil inflammation (EI), ie, eosinophil abscesses, eosinophil surface layering, and surface epithelial alteration) and pathologic features independent of tissue eosinophilia (ie, basal zone hyperplasia [BZH], dilated intercellular spaces, dyskeratotic epithelial cells, and lamina propria fibrosis), we investigated the relationship between blood EoP levels and each pathologic feature assessed by the EoEHSS (Fig 1, E). EoP levels correlated most significantly with measures of eosinophil tissue infiltration (EI composite

REPOSITORY INFORMATION

Global protein composition of the 250- μ g patch

Table E1 presents the allergen levels in extracts used to manufacture the 250- μ g patch. Ara h 3 is the most abundant protein, followed by Ara h 1. Ara h 2 and Ara h 6 represent a minor part of the total protein amount, together about 8% to 15% of all protein. These allergen levels are highly comparable with those found in extracts described in literature (Table E1, right column).^{E1}

Correlation between ISAC IgE and ImmunoCAP IgE determinations

Baseline sera ($n = 51$) were tested for IgE titers for peanut components using both ImmunoCAP and ISAC. ImmunoCAP is available for Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9, whereas ISAC also provides Ara h 6. Fig E1, A, shows the correlation between the sum of IgE titers determined by ImmunoCAP and ISAC, the latter with Ara h 6 IgE included ($R^2 = 0.900$) or excluded ($R^2 = 0.904$). Correlation between IgE to total peanut in ImmunoCAP (ImmunoCAP F13) and the sum of IgE levels to peanut components measured by ImmunoCAP ($R^2 = 0.964$) and ISAC ($R^2 = 0.895$) is shown in Fig E1, B. For individual components present on both platforms, the correlation is shown in Fig E1, C (Ara h 1: $R^2 = 0.784$; Ara h 2: $R^2 = 0.876$; Ara h 3: $R^2 = 0.922$; Ara h 8: $R^2 = 0.792$; Ara h 9: $R^2 < 0.005$). For IgE, ImmunoCAP and ISAC correlated well, except for Ara h 9 for which we found in some sera IgE by ImmunoCAP, but no IgE by ISAC. For components considered as most important allergens (Ara h 2 and Ara h 1), IgE determination of both methods correlate well.

Correlation between ISAC IgG₄ and ImmunoCAP IgG₄ determinations

Sera obtained after 12 months of EPIT with 250- μ g patch ($n = 25$) were analyzed for IgG₄ to peanut components using both ImmunoCAP and ISAC. ImmunoCAP is available for Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9, whereas ISAC also provides Ara h 6. Fig E1, D, shows the correlation between the 2 methods for components present for both methods (Ara h 1: $R^2 = 0.863$; Ara h 2: $R^2 = 0.737$; Ara h 3: $R^2 = 0.7433$; Ara h 8: $R^2 = 0.896$; Ara h 9: $R^2 = 0.908$). For IgG₄, ImmunoCAP and ISAC correlated well for all peanut components.

IgE to peanut components during EPIT

Sera from subjects on active EPIT treatment (250- μ g patch) from time points 0, 3, 6, and 12 months were analyzed for IgE to individual peanut components Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9 using ImmunoCAP (Fig E2). IgE to Ara h 6 was not tested because Ara h 6 is not available on ImmunoCAP. ISAC could not be used to follow IgE evolution during EPIT, because of the disturbing effect of IgG on ISAC. For the main peanut allergens Ara h 1, Ara h 2, and Ara h 3, the IgE titer increased initially and decreased again at 12 months of EPIT, in line with what is often observed for allergen immunotherapy and what was described for the patient population for total peanut IgE.^{E2}

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- E2. Sampson HA, Shreffler WG, Yang WH, Sussman GL, Brown-Whitehorn TF, Nadeau KC, et al. Effect for varying doses of epicutaneous immunotherapy vs placebo on reaction to peanut protein exposure among patients with peanut sensitivity. *JAMA* 2017;318:1798-809.

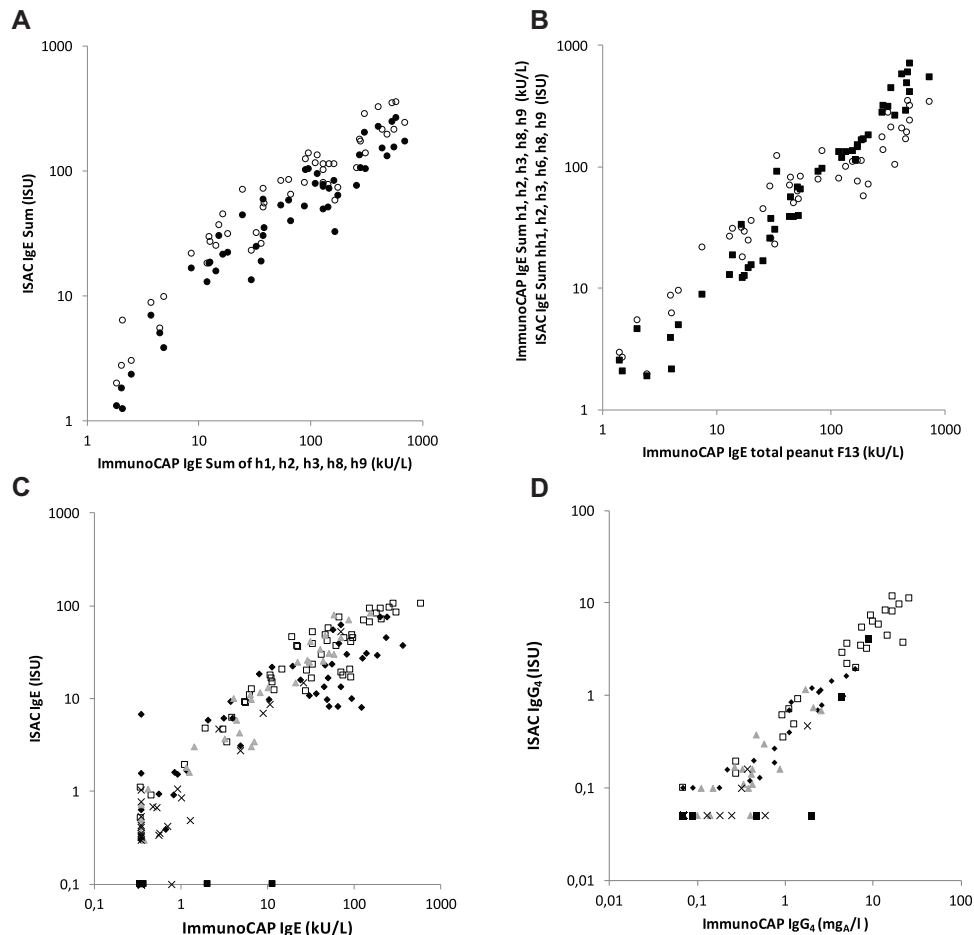


FIG E1. Correlation of ISAC with ImmunoCAP. **A**, Correlation between the sum of IgE titers to peanut components determined by ImmunoCAP and ISAC (sum ISAC excluding Ara h 6: black circles; sum ISAC including Ara h 6: open circles). **B**, Correlation between IgE to total peanut in ImmunoCAP (ImmunoCAP F13) and the sum of IgE to peanut components measured by ImmunoCAP (black squares) and ISAC (open circles). **C**, Correlation of IgE titers to individual peanut components determined by ImmunoCAP and ISAC (Ara h 1: black diamonds; Ara h 2: open squares; Ara h 3: gray triangles; Ara h 8: crosses; Ara h 9: black squares). **D**, Correlation of IgG₄ titers to individual peanut components determined by ImmunoCAP and ISAC (same marker assignment as for Fig E1, C).

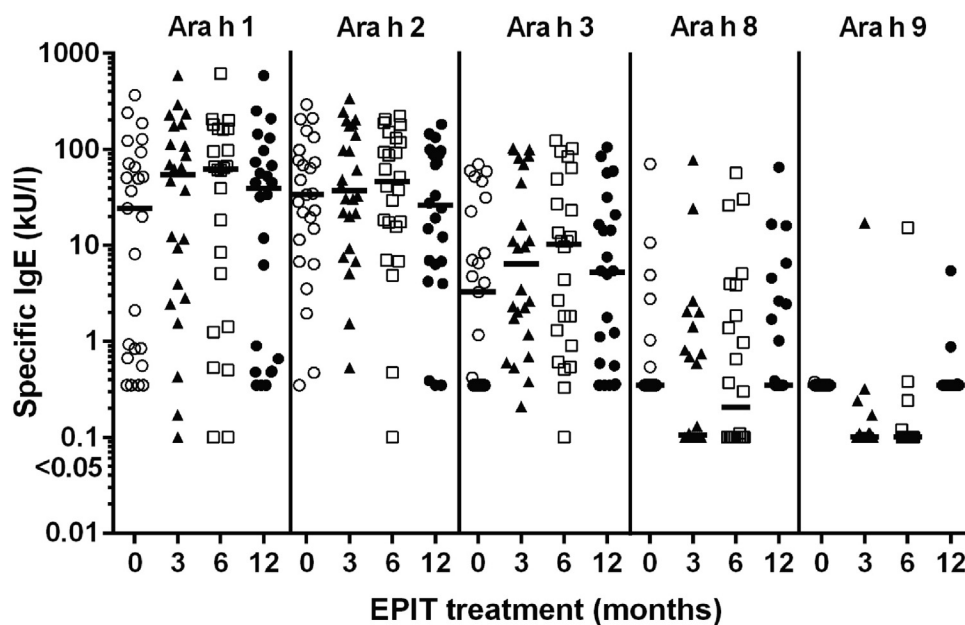


FIG E2. Evolution of IgE to individual peanut components by EPIT with 250- μ g patch. Data obtained with ImmunoCAP. Numbers on the x-axis represent months of EPIT (0 is baseline). Where less than 25 markers per time point are plotted, the markers at less than 0.07 represent multiple markers up to a total of 25. Bars show medians. *Open circles:* baseline; *filled triangles:* 3 months; *open squares:* 6 months; *filled circles:* 12 months.

TABLE E1. Global protein composition of extracts used for the production of the 250-μg patch

Allergen	Range (% of total protein)	
	Extracts for patch (n = 13)	Extracts from literature (n = 20) ^{E1}
Ara h 1	19-28	12-24
Ara h 2	5-9	4-8
Ara h 3	62-72	58-84
Ara h 6	3-6	3-10