

Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy

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Background: Although peanut oral immunotherapy (OIT) has been conclusively shown to cause desensitization, it is currently unknown whether clinical protection persists after stopping therapy.

Objective: Our primary objective was to determine whether peanut OIT can induce sustained unresponsiveness after withdrawal of OIT.

Methods: We conducted a pilot clinical trial of peanut OIT at 2 US centers. Subjects age 1 to 16 years were recruited and treated for up to 5 years with peanut OIT. The protocol was

modified over time to permit dose increases to a maximum of 4000 mg/d peanut protein. Blood was collected at multiple time points. Clinical end points were measured with 5000-mg double-blinded, placebo-controlled food challenges once specific criteria were met.

Results: Of the 39 subjects originally enrolled, 24 completed the protocol and had evaluable outcomes. Twelve (50%) of 24 successfully passed a challenge 1 month after stopping OIT and achieved sustained unresponsiveness. Peanut was added to the diet. At baseline and the time of challenge, such subjects had smaller skin test results, as well as lower IgE levels specific for peanut, Ara h 1, and Ara h 2 and lower ratios of peanut-specific IgE/total IgE compared with subjects not passing. There were no differences in peanut IgG₄ levels or functional activity at the end of the study.

Conclusions: This is the first demonstration of sustained unresponsiveness after peanut OIT, occurring in half of subjects treated for up to 5 years. OIT favorably modified the peanut-specific immune response in all subjects completing the protocol. Smaller skin test results and lower allergen-specific IgE levels were predictive of successful outcome. (*J Allergy Clin Immunol* 2013;■■■:■■■-■■■.)

Key words: Peanut allergy, oral immunotherapy, desensitization, tolerance, sustained unresponsiveness

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Food allergy is the leading cause of anaphylaxis in children, and in the last 20 years, it has become an increasingly prevalent public health problem with adverse medical, psychosocial, and economic effects.¹⁻⁵ This is especially true for peanut allergy, which carries a high risk of severe reactions^{6,7} and is typically a lifelong disorder.^{8,9} Presently, the standard of care for food allergy is strict dietary allergen elimination and ready access to emergency medications. Consensus National Institutes of Health guidelines recommend against the current use of interventional therapies.²

However, recent trials of oral immunotherapy (OIT) have demonstrated progress toward an active treatment approach for food allergy.¹⁰⁻¹⁵ In a preliminary report from an uncontrolled pilot study of peanut OIT in children, our group demonstrated that successful clinical desensitization occurred in 27 (93%) of 29 subjects completing more than 8 months of therapy and was associated with relevant mechanistic changes in the peanut-specific immune response.¹³ Subsequently, a randomized placebo-controlled trial conclusively demonstrated desensitization and immunomodulation, validating the pilot work and supporting the efficacy of OIT in patients with peanut allergy.¹⁵ Other mechanistic studies have shown that peanut OIT complexly

Abbreviations used

DOFC: Desensitization oral food challenge
 FAB: Facilitated antigen binding
 OFC: Oral food challenge
 OIT: Oral immunotherapy
 SOFC: Sustained unresponsiveness oral food challenge
 TF: Treatment failure
 TS: Treatment success

modifies the IgE and IgG₄ responses to the linear epitopes from the major peanut allergens Ara h 1, 2, and 3¹⁶ and induces basophil hyporesponsiveness during treatment.¹⁷ Collectively, these results support the idea that the immunomodulatory effects of OIT are similar to accepted forms of immunotherapy that have been proved to be disease modifying in patients with venom anaphylaxis and respiratory allergy.¹⁸⁻²⁰

Yet only 1 trial to date has conclusively demonstrated that OIT is disease modifying by using egg white powder in subjects with egg allergy.²¹ The term “sustained unresponsiveness” was introduced in this landmark study, describing the ability of a subject to pass an oral food challenge (OFC) after stopping OIT and successfully introduce a previously allergenic food into the diet *ad libitum*. Whereas egg allergy is commonly outgrown, this is uncommon for peanut allergy, and sustained unresponsiveness to peanut has not previously been shown.

We sought to determine, in the same peanut-allergic cohort in whom desensitization was previously reported,¹³ whether long-term treatment with OIT would result in sustained unresponsiveness to peanut and to identify the clinical and immunologic parameters associated with this state.

METHODS**Subject recruitment**

This trial was conducted in accordance with the principles of the Declaration of Helsinki. Ethics approval was obtained through the Institutional Review Boards at Duke University Medical Center and the University of Arkansas for Medical Sciences. Written informed consent was obtained before study participation in accordance with each institution's ethics guidelines for research in children. Subjects (age, 1-16 years) were recruited from the allergy and immunology clinics or surrounding community physicians' offices at both sites. An interim analysis of this cohort of subjects was previously published¹³; the end-of-study results of the same clinical trial are presented here.

OIT protocol

Details of subject selection and the peanut OIT protocol have been previously published¹³ and are available in the [Methods](#) section in this article's Online Repository at www.jacionline.org. Briefly, OIT was administered in an open-label fashion to subjects with peanut allergy daily in 3 phases (ie, initial-day escalation, build-up, and maintenance), which continued until subjects met eligibility for end point assessment, as described below. For the duration of the study, subjects strictly avoided all peanut except for that provided in the dose of their study product.

Clinical end points

Subjects in this study underwent at least 3 OFCs. The first, which was previously reported,¹³ was an open OFC to 3900 mg of peanut protein conducted shortly after reaching the maintenance dose. In the current study qualifying subjects from the previous report were evaluated with

2 double-blind, placebo-controlled food challenges to a total of 5000 mg of peanut protein performed 4 weeks apart. Details of these challenges have been previously published.¹⁵ The first of these challenges (referred to in the figures as desensitization oral food challenge [DOFC]) was performed to assess reactivity while receiving treatment, and OIT was stopped if this double-blind, placebo-controlled food challenge was passed. The next challenge (referred to in the figures as sustained unresponsiveness oral food challenge [SOFC]) was conducted 4 weeks after stopping OIT and assessed the primary end point called sustained unresponsiveness, which we operationally defined as the ability to asymptotically consume all of the challenge material and then an open oral feeding of one serving (eg, 8,000-10,000 mg) of peanut butter afterward on the same day. Subjects passing the SOFC were classified as treatment successes (TSs), and those with convincing allergic symptoms during their final SOFC or open feeding were classified as treatment failures (TFs). The criteria for the timing of the assessment of sustained unresponsiveness varied as the study progressed. The initial protocol called for SOFC once peanut IgE levels were less than 2 kU/L. We subsequently amended the protocol to offer SOFCs to subjects with a peanut IgE level of less than 15 kU/L, a peanut skin prick test response of less than 5 mm, and no peanut-related reactions in the previous 6 months. Because of the exploratory nature of this pilot study, if subjects failed the SOFC during these first 2 phases of evaluation, they resumed OIT. The final phase of assessment for sustained unresponsiveness occurred in all remaining subjects who underwent SOFC at the completion of 5 years of OIT, regardless of their immune parameters. TSs were advised to incorporate peanut into the diet *ad libitum* at least several days per week. The day after the final SOFC, TFs were restarted on a predetermined amount of a peanut-containing food daily and are being followed.

Clinical and mechanistic studies

Skin prick tests were performed in standard clinical fashion throughout the study. Mechanistic studies investigating serologic and cellular responses to OIT and using purified peanut reagents were performed, as previously described,¹³ on the subjects enrolled at one of the study sites because of the availability of specimens there. Additional details about these assays can be found in the [Methods](#) section in this article's Online Repository.

Follow-up

A 10-question telephone survey was developed to assess post-OIT dietary habits, safety, and beliefs/attitudes after study completion. Contact was attempted with all subjects who had an evaluable outcome. The questionnaire is available in the [Methods](#) section in this article's Online Repository.

Statistical methods

We computed averages, variances, frequencies, proportions, and graphic displays for all clinical and immunologic variables (GraphPad Software, La Jolla, Calif). We used Wilcoxon rank sum and Mann-Whitney tests for between-group comparisons of immunologic and facilitated antigen binding (FAB) data, respectively, at single time points. Kruskal-Wallis and Fisher exact tests were used for between-group comparisons of questionnaire data. For longitudinal analyses, we used Bonferroni-corrected, nonparametric, 2-way, repeated-measures ANOVA or simple linear regression. The area under the receiver operating curve was calculated to determine between-group predictors. *P* values of less than .05 were considered significant.

RESULTS**Subjects' demographics**

Thirty-nine subjects were originally enrolled in the trial, and ultimately, 24 (62%) had an evaluable outcome with respect to sustained unresponsiveness ([Fig 1](#)). Six (15%) of 39 enrolled subjects withdrew for allergic side effects; the remaining 9 withdrew for personal or other reasons. Clinical and demographic

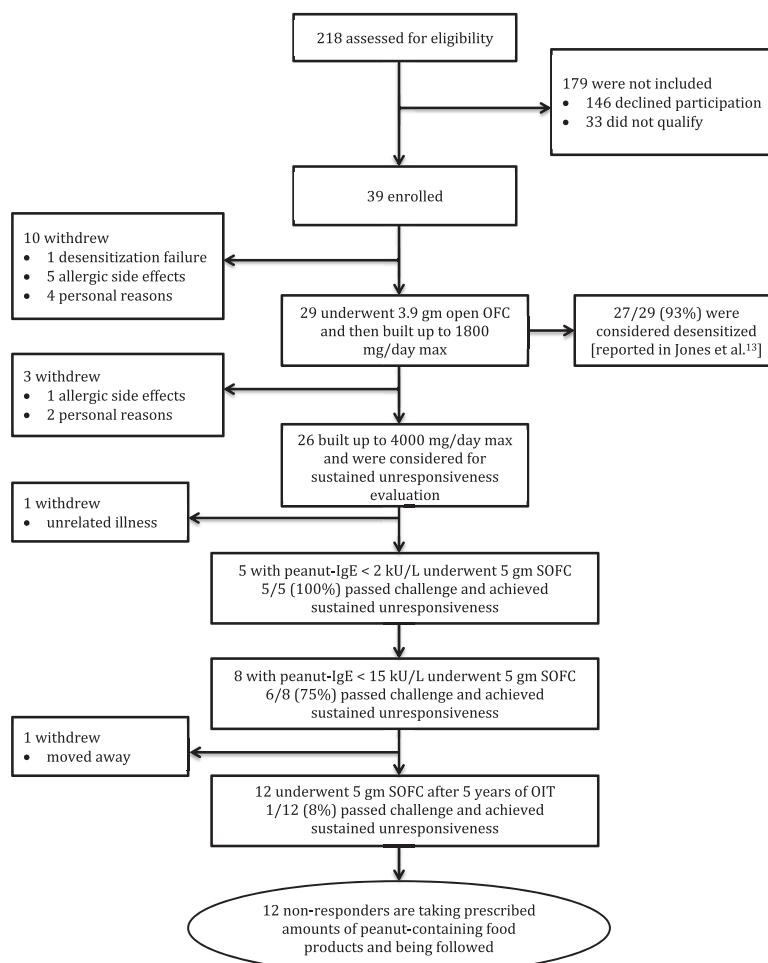


FIG 1. Conduct of the study.

characteristics of the 24 evaluated subjects were no different than those of the subjects withdrawing (not shown). As previously noted, subjects in this study were not evaluated for sustained unresponsiveness at the same time interval, with a mean length of treatment of 1453 days (SD, 663 days).

Half of finishing subjects achieved sustained unresponsiveness

Twelve TSs (50% per protocol or 31% by intent to treat) consumed 5000 mg of peanut protein and an open oral feeding of peanut butter without symptoms 4 weeks after stopping OIT and were considered to have achieved sustained unresponsiveness (Fig 2). Among TFs, the median amount of peanut protein ingested cumulatively before the development of symptoms was 3750 mg (range, 1500-5000 mg), which is equivalent to approximately 12 peanuts on average.

Sustained unresponsiveness was inversely associated with skin test reactivity at baseline and end of study

At baseline, TSs had smaller skin test results than TFs (median, 9 vs 14 mm, respectively; $P = .02$; Fig 3, A). During treatment in all subjects, OIT suppressed mast cell

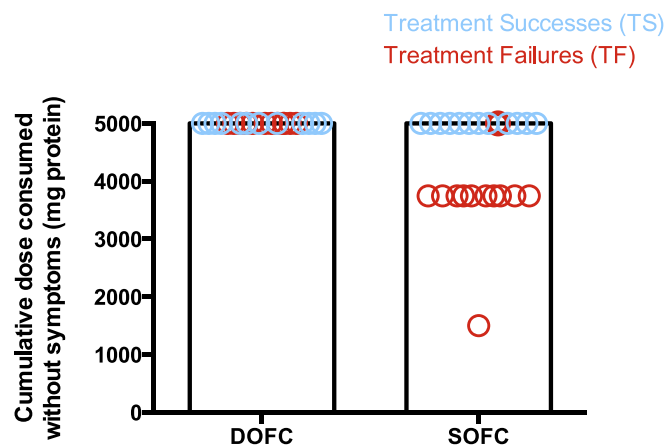


FIG 2. Food challenge results. Shown are the cumulative amounts of protein successfully ingested before onset of symptoms in TS (blue circles) and TFs (red circles). Each circle represents 1 subject.

responsiveness, as demonstrated by a reduction in mean wheal diameter on skin prick tests performed at baseline and at the time of the DOFC. This suppression persisted in TSs on discontinuation of OIT, whereas in TFs wheal diameters returned to near-baseline levels (Fig 3, B).

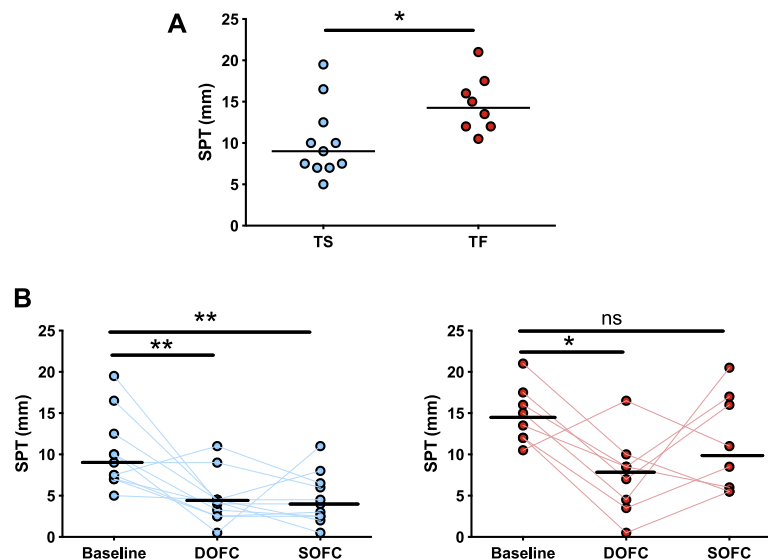


FIG 3. Skin prick test (SPT) results. **A**, Average mean wheal diameters at baseline by outcome. **B**, Mean wheal diameters over time by outcome. Lines represent median values. * $P < .05$ and ** $P < .01$. ns, Not significant.

Intensity of allergic priming was inversely related to sustained unresponsiveness

Compared with TFs at baseline and at every subsequent time point measured, TSs had lower median IgE levels specific for peanut, Ara h 1, and Ara h 2 (Fig 4, A and B). The total amount of IgE was not different between groups (data not shown). Importantly, all subjects receiving OIT experienced a longitudinal reduction to less than baseline levels for some (peanut or Ara h 1, 2, or 3) but not all (Ara h 8 or 9) allergen-specific IgEs (Fig 4, C). All subjects experienced a reduction in the peanut-specific IgE/total IgE ratio (Fig 4, D), and this ratio was less at baseline and at the end of the study in TSs compared with that seen in TFs (Fig 4, E).

Sustained unresponsiveness was not associated with amount or inhibitory capacity of peanut-specific IgG

Levels of peanut-specific IgG, including IgG₄, increased in all subjects (Fig 5, A). During the first year, a striking increase occurred in the total amount of peanut-specific IgG of subtypes other than IgG₄. This production peaked at approximately 12 months of therapy and then decreased, reaching a steady state at around 18 months; in contrast, IgG₄ production steadily increased over time. The amount of peanut-specific IgG₄ was not associated with clinical outcome at baseline or at the end of the study (Fig 5, B). The peanut-specific IgE/IgG₄ ratio steadily decreased over the course of therapy (Fig 5, C) and was significantly different between groups at baseline and at the end of the study (Fig 5, D). Fig 6, A, shows the inhibitory capacity of IgG in a subset ($n = 14$) of subjects, demonstrating considerable variance but an overall increase in the inhibitory activity of subjects' sera over time (slope, -0.49 [95% CI, -0.78 to -0.19], $P = .002$). Unexpectedly, there was a trend toward TS sera having lower inhibitory function than TF sera, which was statistically significant at 12 months but not at the time of challenge (Fig 6, B).

Sustained unresponsiveness was not associated with regulatory T-cell numbers at SOFC

There were no between-group differences in the proportion of CD4⁺CD25⁺ forkhead box protein 3–positive T cells at the end of the study in a subset of subjects for whom sufficient cells were available (see Fig E1 in this article's Online Repository at www.jacionline.org).

Predictors of sustained unresponsiveness

We determined with receiver operating characteristic analysis whether readily available parameters could discriminate between TSs and TFs at baseline and the end of the study. At baseline, the strongest predictor was the peanut-specific IgE/total IgE ratio (area under the curve, 0.96; $P = .005$; see Fig E2 in this article's Online Repository at www.jacionline.org), whereas at the end of the study, it was the Ara h 2–specific IgE level (area under the curve, 0.9; $P = .003$; see Fig E3 in this article's Online Repository at www.jacionline.org). Notably, the second-best predictor in both baseline and end-of-study parameters was the peanut-specific IgE level. Peanut-specific IgG₄ had no discriminatory power at either time point.

TSs consume peanut without symptoms after stopping OIT

Questionnaire response was 87.5% ($n = 21$). The median time to follow-up after the SOFC in the TF group was 12 months (range, 3–36 months), and that among TSs was 40 months (range, 3–56 months). None of the TSs reported allergic reactions associated with peanut exposure, whereas 3 (14%) TFs reported mild peanut reactions, none of which required epinephrine or a physician's office visit. TSs incorporated a median 555 mg/d (range, 0–4000 mg/d) peanut protein into the diet on average 3 days per week (range, 0–7 days per week). This was less frequent compared with TFs,

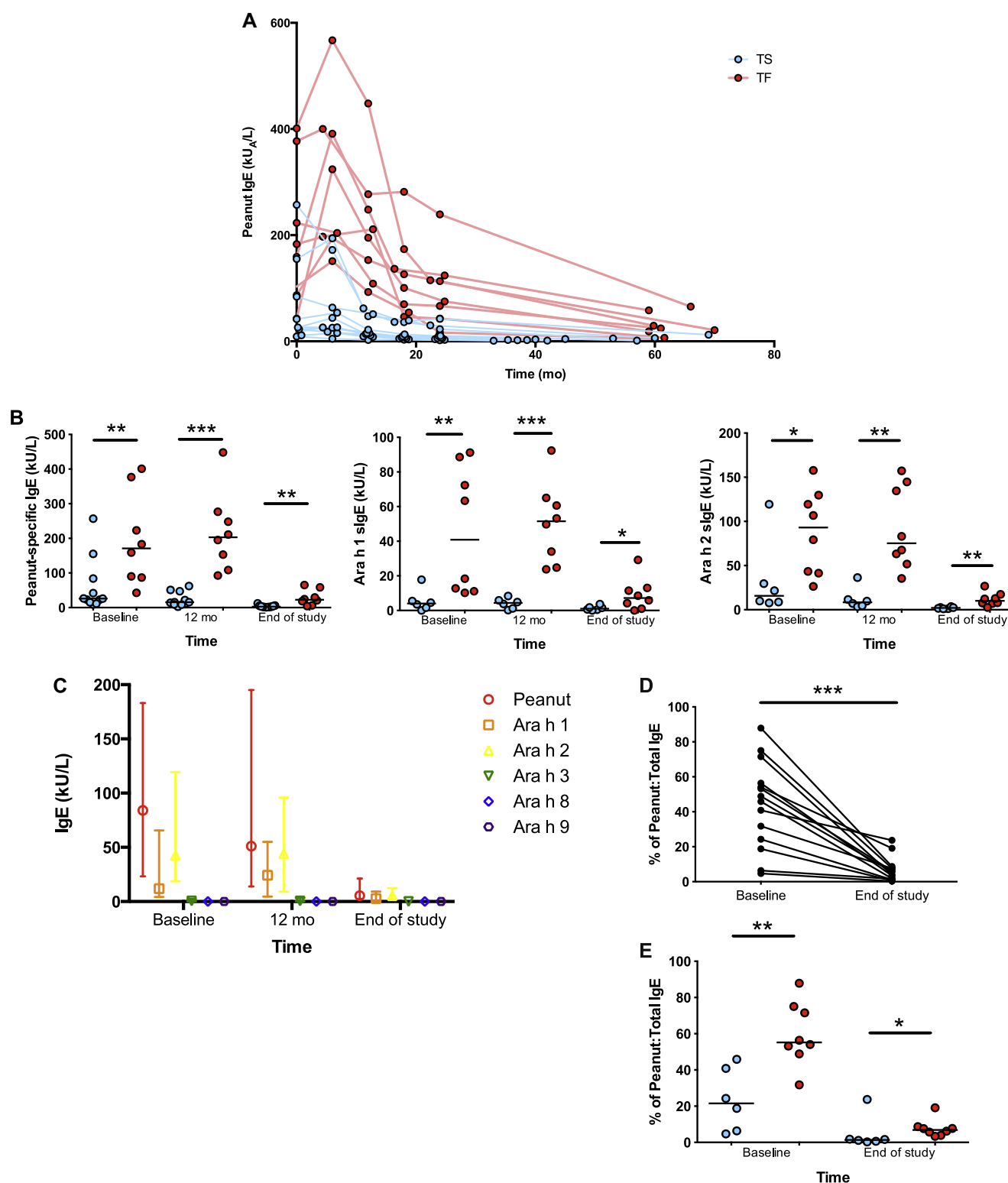


FIG 4. Peanut- and component-specific serum IgE levels during OIT. **A–C**, Longitudinal peanut-specific (Fig 4, **A**) and Ara h 1- and Ara h 2-specific (Fig 4, **B**) IgE levels are shown for individual subjects by outcome and for all components in all subjects (Fig 4, **C**). **D** and **E**, Peanut-specific IgE/total IgE ratio is shown for all subjects (Fig 4, **D**) and for individual subjects by outcome (Fig 4, **E**). All point estimates are medians with interquartile ranges (Fig 4, **C**). * $P < .05$, ** $P < .01$, and *** $P < .001$. *ns*, Not significant.

who incorporated a median of 895 mg/d (range, 330–4000 mg/d) peanut protein 7 days per week (range, 5–7 days per

week; $P = .0003$ for frequency comparison). The longest median interval in days between peanut exposures was greater

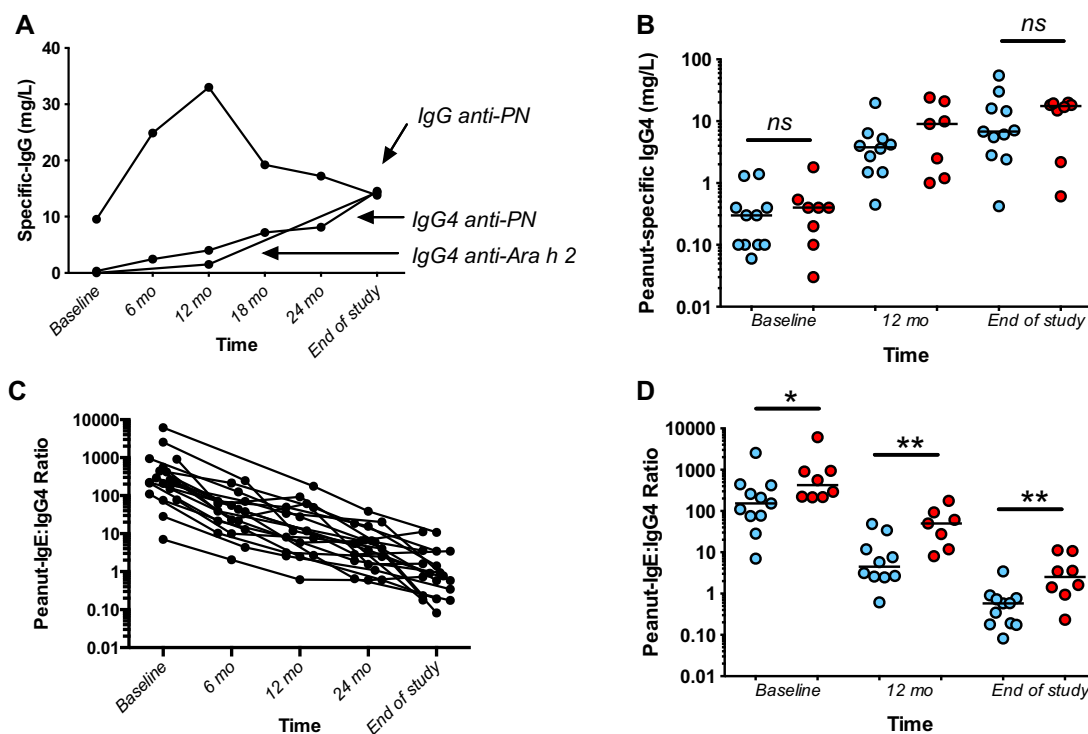


FIG 5. Peanut-specific serum IgG levels, IgG₄ levels, and IgE/IgG₄ ratios during OIT. **A**, Median values for all subjects' longitudinal peanut-specific IgG and IgG₄ and Ara h 2-specific IgG₄ levels are shown. **B**, Peanut-specific IgG₄ levels are shown for subjects by outcome. **C** and **D**, Peanut-specific IgE/IgG₄ ratios are shown for all subjects (Fig 5, C) and by outcome (Fig 5, D). Lines represent medians. * $P < .05$ and ** $P < .01$. ns, Not significant.

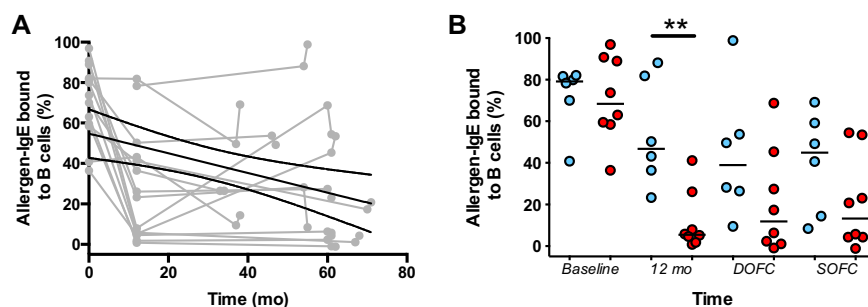


FIG 6. Serum from subjects receiving peanut OIT inhibits FAB. **A**, FAB data are shown for all subjects in gray, with the mean linear regression slope indicated by the black line and 95% CIs indicated by the hatched line. **B**, FAB by outcome is shown, with lines representing medians. ** $P < .01$.

in TSs (14; range, 2->365) than in TFs (2; range, 0-7; $P = .004$). One of the TSs stopped taking peanut in the diet because of personal and not medical considerations, and over the subsequent year, we observed significant increases in his skin test size (0.5-16 mm) and *in vitro* IgE levels (3.56-11.5 kU/L). We have advised him to continue peanut avoidance. Fifty-seven percent of parents encountered some difficulty in getting their child to willingly consume peanut in their diets, with 19% each reporting difficulty "about half of the time," and "every time"; these frequencies were not different between TSs and TFs. All surveyed families reported satisfaction with their decision to participate in the study; their poststudy narrative comments appear in Table E1 in this article's Online Repository at www.jacionline.org.

DISCUSSION

Here we report on the end-of-study results of our initial pilot trial of peanut OIT, providing an update from our previously published interim report.¹³ After a maximum of 5 years and 4000 mg/d OIT, all subjects completing the study successfully ingested 5000 mg of peanut protein without symptoms during the DOFC and were considered desensitized. Fifty percent of these subjects successfully repeated this challenge 1 month after stopping OIT and demonstrated sustained unresponsiveness. This trial lacked the placebo control required for definitive proof, but on the basis of natural history studies,^{8,9,22} it is highly unlikely that half of the study population would have outgrown peanut allergy during this study period. Thus these data represent the first descriptions of a lasting state of sustained unresponsiveness among children with

peanut allergy treated with OIT and serve as proof of concept that such an outcome is possible. We advised successful subjects to incorporate peanut into their diets on a regular basis without restriction, as if they were no longer allergic. A relapse appears likely in the 1 successful subject described above who could not do this. However, despite reporting that it was frequently difficult, all of the other TSs now regularly consume relatively small amounts of peanut to which they were previously highly allergic. It is not known at present whether this clinical change is permanent, but notably, we have a median of more than 40 months' follow-up. The ability of OIT trial participants, even those classified as successes, to reintroduce allergen into the diet long term after study completion has been questioned,²³ and it appears this might be necessary to prevent relapse in some subjects, as we observed. Although we report a substantially higher proportion of dietary adherence than Keet et al²³ over a longer period of follow-up, we share the concern that a need for ongoing regular oral exposure during and after therapy might be a potential limitation of this approach and an area that requires much more study.

Interestingly, among TFs, a median cumulative dose of 3750 mg was required to elicit symptoms during the SOFC. This eliciting dose is several orders of magnitude greater than at baseline, when, although not formally challenged, all subjects had clinical reactions before reaching the 50-mg dose on the initial escalation day.¹³ Thus even in participants not achieving the primary outcome, OIT still induced a state of altered reactivity between peanut allergy and sustained unresponsiveness. One possible explanation is that among TFs, who all passed the DOFC and were considered desensitized, the protective effects of OIT began to subside during the 4 weeks off therapy. As was seen in another short peanut OIT trial,¹⁴ clinical reactivity in the posttreatment OFC was linked to the return of peanut-induced mast cell activation. Other studies of egg, milk, and peanut OIT have also shown that the clinical desensitization effect was transient, in some cases within 1 or 2 weeks after discontinuing OIT.^{11,14,24} However, as in this study, the reaction threshold in all subjects from both of these studies remained well above baseline values. Of note, subjects in the current study were treated for up to 5 years, the longest trial of peanut OIT published to date and far longer than the studies cited above. This could help explain the overall success rate and also the high eliciting dose during the SOFC among TFs. On the basis of other OIT studies and the broad experience with subcutaneous allergen immunotherapy, it is likely that the length of treatment^{25,26} and the antigen dose²⁴ influence the durability of the treatment effect. However, the factors governing the change in clinical state remain poorly understood and require further study. These observations also underscore the importance of study design with respect to how and when the primary outcome is defined and measured.

Mechanistically, we looked for evidence of increased peanut-specific regulatory T-cell numbers in TSs, but these cell frequencies did not differ by outcome. We observed that an overall increase in peanut-specific IgG₄ levels during OIT was accompanied by a consistent parallel decrease in allergen-IgE binding to B cells. Both increased specific IgG₄ levels and increased serum inhibitory activity were paradoxically observed in TFs compared with TSs. One explanation could be the use of pooled indicator serum rather than participants' autologous baseline serum for the IgE-FAB inhibition assay. The availability

of serum was insufficient to allow for this alternative experimental design. However, we consider this explanation unlikely because in previous studies use of indicator or baseline autologous serum has yielded comparable results,²⁷ and the data are consistent with previous studies of venom immunotherapy in which these markers bore no relationship to clinical outcome.²⁸ During peanut OIT, in contrast to immunotherapy for respiratory allergy,²⁹ it is possible that IgE and/or mast cell suppression drives desensitization rather than increased IgG₄ and IgE-FAB inhibition. These findings warrant further study.

We observed a number of other interesting findings about the antibody response during OIT. In TFs the peanut-specific IgE level amplified over the first 6 months of therapy, as did peanut IgG responses (including IgG₄ and other isotypes). Because TFs started OIT with higher baseline peanut-specific IgE levels than TSs, this amplification could be due to IgE-mediated facilitated antigen presentation, which has been shown to drive pathologic T-cell responses in subjects with peanut allergy but not control subjects.³⁰ If true, this could explain why subjects with lower IgE levels at baseline have better outcomes after OIT. The possible role of IgE-mediated facilitated antigen presentation in determining the response to OIT deserves further study. An important aspect of the study is that in all subjects, whether initially amplified or not, peanut-specific IgE levels were reduced to less than baseline levels after a median of 12 months of OIT, and this occurred proportionally in both groups to the same degree and independent of outcome (data not shown). TSs had lower IgE levels to peanut, Ara h 1, and Ara h 2 at baseline and the end of the study compared with TFs, a trend observed in numerous other natural history and OIT studies. The change over time in total peanut-specific IgE levels was paralleled by similar changes in IgE specific for Ara h 1 and 2 but not Ara h 3, 8, and 9, suggesting that IgE binding to these major allergens is an important target during OIT. Although we can detect Ara h 1 and Ara h 2 in the peanut flour used in this study (data not shown), in general, there is little known about the relationship between major allergen dose and outcomes. This is a knowledge gap requiring further investigation.

Several single-center uncontrolled studies have previously reported on the development of sustained unresponsiveness after egg^{11,26} and milk^{11,24} OIT but none with peanut. The only report of sustained unresponsiveness from a placebo-controlled trial of food OIT was recently published in a landmark egg OIT paper from the Consortium of Food Allergy Research.²¹ This large multicenter study by Burks et al²¹ demonstrated that sustained unresponsiveness occurred in 27.5% of subjects actively treated for 22 months compared with none in the placebo group. Successes consumed 10 g of egg white powder and 1 whole egg without symptoms during a challenge 4 to 6 weeks after stopping OIT. Consistent with our observations, sustained unresponsiveness was associated with significantly smaller skin prick test responses at the time of the SOFC, linking sustained mast cell suppression to a successful outcome. Importantly, no OIT study to date, including the current one, has followed a placebo control group through to the end of the study.

Nonconsecutive enrollment, substantial subject withdrawal, and the lack of a control group are limitations in this trial, but they are common to exploratory pilot studies that carry significant and unknown risks on inception. It is worth noting that although there are currently many trials of peanut OIT around the world, this trial began in 2003 and was one of the first.

Recruitment and retention were problematic, and safety concerns were paramount.

In summary, for the first time, we demonstrate that sustained unresponsiveness developed in half of subjects with peanut allergy able to complete treatment with years of OIT. Although it is not proved that this was due to OIT, it is unlikely to have occurred spontaneously. This change in reactivity has allowed the treatment successes to resume a diet unrestricted of peanut. Although OIT was immunomodulatory in all participants, lower peanut-specific IgE levels at baseline and smaller skin test responses at the end of the study predicted successful outcomes. Larger trials with rigorous designs are warranted to further investigate the long-term effects of OIT in subjects with peanut allergy.

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Key messages

- Half of subjects with peanut allergy completing this pilot study of peanut OIT consumed 5000 mg of peanut protein and an open oral feeding of peanut butter 1 month after stopping treatment.
- It is not known whether this change is permanent, but most of these subjects are consuming peanuts in their diet.
- These subjects started and ended the study with less peanut-, Ara h 1-, and Ara h 2- specific IgE levels; smaller skin test results, lower peanut-specific IgE/total IgE ratios; and similar IgG₄ levels compared with those unable to pass the final challenge.

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METHODS

Subject selection

Included subjects had a clinical history of reaction to peanut within 60 minutes of ingestion, a positive peanut skin prick test response (≥ 3 mm larger than that elicited by the negative control), and a peanut CAP-FEIA result of 15 kU/L or greater (Thermo Fisher, Uppsala, Sweden). Subjects were also included if they had a CAP-FEIA result of 7 kU/L or greater and a clinical reaction within the previous 6 months. Clinical anaphylaxis was not an exclusion criterion unless it was deemed severe or life-threatening (eg, involving hypotension). Other exclusions included severe or poorly controlled asthma or a medical condition preventing food challenge.

OIT protocol phases

Initial-day escalation phase. The initial-day escalation phase was undertaken at the research unit at each institution, with appropriate resuscitation medications at the bedside. Dosing began at 0.1 mg of peanut protein, followed by an approximate doubling every 30 minutes, up to 50 mg of cumulative protein. The highest tolerated single dose was the starting dose for the build-up phase, which was initiated in the research unit the following day.

Build-up phase. Subjects were instructed to ingest the daily dose of peanut protein at home with other safe foods in 2 or 3 bites. Doses were increased 25 mg every 2 weeks to 300 mg. Subjects returned to the clinic for dose escalations. For subjects who stopped dosing at less than 50 mg on the initial-day escalation, their doses were doubled every 2 weeks until they reached 50 mg, and then the increases were 25 mg. Build-up dosing was delayed if subjects had evidence of illness (eg, viral infection) at the time of scheduled up dosing^{E1}; therefore the time to reach maintenance dosing varied between subjects.

Maintenance phase. After reaching 300 mg of peanut protein daily, subjects continued this dose until clinical desensitization was measured with the 3900-mg OFC previously reported.^{E2} After this OFC, subjects were increased to a daily OIT dose of 1800 mg if the peanut-specific IgE levels remained greater than 2 kU/L after 12 months on a maintenance dose; this escalation occurred in all subjects reported. Subsequently, subjects were escalated to a top maintenance dose of 4000 mg/d peanut protein. All subjects began the study taking peanut protein (from Partially Defatted Peanut Flour 12% Fat Light Roast, Golden Peanut Company, Alpharetta, Ga; 2 g of flour = 1 g of peanut protein). Doses were premeasured and mixed in a food vehicle of the subject's choosing and taken over 2 or 3 bites. At the University of Arkansas for Medical Sciences, the protocol was modified to permit the transition from study protein to a "food equivalent" (eg, peanut M&Ms and peanut snack bars) using defined foods at equivalent doses of peanut protein to continue maintenance OIT dosing after the initial DOFC in year 1.

Skin prick testing

Skin prick tests (1:20) with standard peanut extract (Greer Laboratories, Lenoir, NC) and saline and histamine controls were performed at enrollment, after 4 months of maintenance therapy, and every 4 months thereafter. Wheal size was the average of the largest diameter and the perpendicular midpoint diameter. Measurements were tape transferred to the paper source document.

Mechanistic methods

Assays for IgE, IgG, IgG₄, IgA₁, and IgA₂. Peanut-specific IgE, IgG, and IgG₄ levels were quantified in serum samples by using the ImmunoCAP 100 instrument (Phadia AB, Uppsala, Sweden), according to the manufacturer's instructions. Total IgE; component-specific IgE anti-Ara h 1, 2, 3, 8, and 9; and Ara h 2-specific IgG₄ levels were also quantified on the ImmunoCAP 100 instrument. Peanut-specific IgA₁ and IgA₂ levels in serum were measured by means of ELISA with a similar protocol, as described for IgA in saliva.^{E3} Briefly, plates were coated with 20 μ g/mL peanut protein extract for 1 hour and then blocked with a 2% BSA solution. For IgA₁, serum samples were diluted 1:250; for IgA₂, serum was diluted 1:50 and

incubated overnight at 4°C. IgA₁ was detected with clone B3506B4 (Southern Biotech, Birmingham, Ala), and IgA₂ was detected with clone A9604D2 (Southern Biotech); both are directly conjugated to horseradish peroxidase enzyme. Values are reported as optical density measured at 450 nm (OD₄₅₀).

IgE-FAB assay. FAB analysis was conducted with a similar protocol, as previously described.^{E2} Briefly, high peanut-specific IgE-containing indicator serum (from the laboratory of A.W.B.) was incubated with 0.3 μ g/mL peanut extract in the presence of sera obtained before OIT (0 months), after 12 and 36 months of OIT, and 1 month after OIT withdrawal. The indicator serum contained pooled sera from 6 unrelated subjects with peanut allergy, with a final peanut-specific IgE level of 335 kU/L quantified by using ImmunoCAP. Results are shown as the percentage of allergen-IgE complexes bound to B cells, as detected by using flow cytometry.

Secreted cytokine assay. PBMCs were isolated from heparinized blood by using Ficoll-based density separation (LymphoH; Atlanta Biologicals, Lawrenceville, Ga). Suspended PBMCs were distributed into 96-well flat-bottom plates at a concentration of 4×10^5 cells per well in triplicate and incubated with crude peanut protein (200 μ g/mL), concanavalin A (40 μ g/mL; Sigma, St Louis, Mo), or medium alone (RPMI-1640 with 2 mmol/L L-glutamine, 25 mmol/L HEPES buffer containing 10% human AB serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin; Mediatech, Manassas, Va). Cells were cultured at 37°C in a 5% CO₂ humidified atmosphere for 96 hours. Culture supernatants were collected and analyzed in duplicate for IL-5 and IL-13 by means of ELISA (R&D Systems, Minneapolis, Minn).

Regulatory T-cell assay. PBMCs (2×10^6 cells per well) were cultured in 24-well plates under the same stimulation conditions described for cytokine analyses. After 6 days, cells were collected and stained with fluorescent mAbs: anti-CD3-PerCP, CD4-fluorescein isothiocyanate, and CD25-phycoerythrin (BD Biosciences, San Jose, Calif). Additional intracellular staining with anti-forkhead box protein 3-allophycocyanin was carried out after fixation/permeabilization of the cells (eBioscience, San Diego, Calif). Isotype controls were included for each condition. The samples were run for 4-color detection in a FACSCalibur flow cytometer (Beckman Coulter, Fullerton, Calif). At least 10,000 events were acquired for each experimental condition, and data were analyzed with FlowJo software (TreeStar, Ashland, Ore).

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FOLLOW-UP QUESTIONNAIRE

Study participant: _____

A suggested introduction by the caller: Thank you again for being one of the very first to volunteer for our peanut study and for agreeing to speak to us today. In this short survey we are hoping to find out more about what has happened since you participated in your last food challenge, so that we may learn more about the long-term effects of treatment. Please note there are no "right" or "wrong" answers – simply try to answer these questions to the best of your ability.

1. How many months has it been since the open food challenge?

2. What form or forms of peanut does your child ingest?
3. How much (quantity) peanut does your child ingest?
4. How often does your child eat peanut?
5. What is approximately the longest interval of time (days, weeks, months) that your child has gone without eating peanut since the open challenge?
6. For the next question, please select the best answer regarding your child's willingness to take peanut in his/her diet. How often do you encounter resistance or difficulty in getting your child to eat peanut?
 - a) not at all
 - b) occasionally
 - c) about half of the time
 - d) almost always
 - e) every time
7. Has your child had any clinical symptoms/reactions with peanut ingestion since the end of the study? How many times? Did any of them require treatment? Evaluation by a doctor?
8. Has your participation in the study affected the way you manage your child's epinephrine autoinjector for his/her peanut allergy (best answer)?
 - (a) No, he/she remains allergic to other foods
 - (b) No, we still have it ready all the time
 - (c) No, but I wonder if we still need it
 - (d) Yes, I am much less strict about it
 - (e) Yes, we no longer really carry one
9. Do you consider your child 'cured' of their peanut allergy?
10. If you had to do it over again, would you participate in this study? Yes/ No? Please explain why.

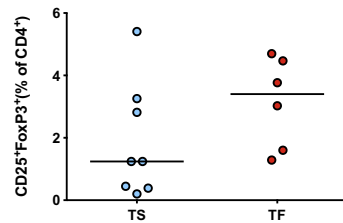
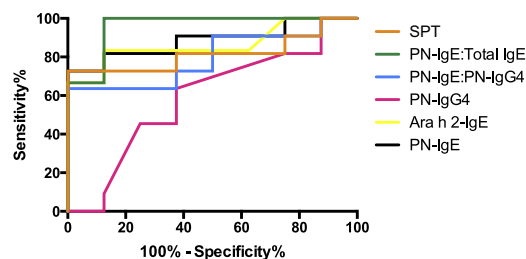
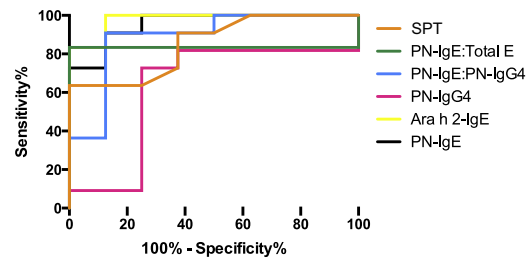


FIG E1. Regulatory T-cell numbers are not statistically different between the TS and TF groups at the time of SOFC. CD4⁺CD25⁺ forkhead box protein 3 (*FoxP3*)⁺ regulatory T cells are shown as a percentage of CD4⁺ T cells. Regulatory T-cell numbers were measured from PBMCs cultured with peanut proteins at the time of SOFC.



	Value	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio	AUC
PN-IgE:Total IgE	< 47.4	100	54.1% to 100%	87.5	47.4% to 99.7%	8	0.96
PN-IgE	< 85.5	81.8	48.2% to 97.7%	87.5	47.4% to 99.7%	6.55	0.89
Ara h 2-IgE	< 35.5	83.3	35.9% to 100%	87.5	47.4% to 99.7%	6.67	0.86
SPT	< 11.3	72.7	39% to 93.9%	87.5	47.4% to 99.7%	5.82	0.82
PN IgE: PN IgG4	< 215.8	63.6	30.8% to 89%	87.5	47.4% to 99.7%	5.09	0.80
PN IgG4	< 0.2	45.5	16.8% to 76.6%	75	34.9% to 96.8%	1.82	0.59

FIG E2. The discriminative potential of baseline factors on OIT outcome. Receiver operating characteristic curves are shown in the figure. Optimal cut points for each factor are shown in *tabular form* below in descending order by area under the curve (AUC). *PN*, Peanut; *SPT*, skin prick test.



	Value	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio	AUC
Ara h 2-IgE	< 5.4	100	54.1% to 100%	87.5	47.4% to 99.7%	8	0.98
PN-IgE	< 6.4	90.9	58.7% to 100%	87.5	47.4% to 99.7%	7.27	0.95
PN-IgE: PN-IgG4	< 0.9	90.9	58.7% to 100%	87.5	47.4% to 99.7%	7.27	0.89
SPT	< 5.8	63.6	30.8% to 89.1%	75	34.9% to 96.8%	2.55	0.85
PN-IgE:Total IgE	< 3.7	83.3	35.9% to 99.6%	87.5	47.4% to 99.7%	6.67	0.83
PN-IgG4	< 14.7	72.7	39% to 94%	75	34.9% to 96.8%	2.91	0.63

FIG E3. The discriminative potential of end-of-study factors on OIT outcome. Receiver operating characteristic curves are shown in the figure. Optimal cut points for each factor shown in *tabular form* below in descending order by area under the curve (AUC). *PN*, Peanut; *SPT*, skin prick test.

TABLE E1. Answers to question 10 of the follow-up questionnaire

10 (Yes/no)	10 (Why?)
Yes	We feel that our child is able to liberalize diet; is less anxious at school; we feel that our child will be safer in case of accidental exposure
Yes	Our child is safe from some accidental exposure
Yes	Our child will be safer in case of accidental ingestion; we are less anxious about starting school/visiting people's houses; we feel that we have access to a support system through the study coordinators
Yes	It has helped us feel safer now in case something were to happen accidentally; we have a better understanding of being able to recognize symptoms and treat a reaction due to excellent teaching and support from study coordinators
Yes	Our child is safer now and can go to school without us being concerned
Yes	Our child is safer in the setting of an accidental exposure; and this has really helped his sensitivity
Yes	Made an amazing difference in our life; our child is cured of their allergy; we don't have to read labels
Yes	We feel that he is safe and protected just in case he got exposed to something
Yes	It has given us peace of mind; we like having the study coordinator support
Yes	It has definitely given us peace of mind
Yes	We feel safe if there were an accidental exposure; and it has improved our quality of life
Yes	We feel safer than before in case there were to be some sort of accidental ingestion
Yes	We really feel protected in case of accidental exposure since being in the study
Yes	Our quality of life has improved; we no longer live in fear; we feel protected in case of accidentally coming in contact with a peanut product
Yes	Her quality of life is now normal; if we did not do this, her life would have been sheltered
Yes	We feel protected in case there were to be an accidental exposure