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A Phase 1 Study of Heat/Phenol Killed, E. coli-Encapsulated, Recombinant Modified Peanut Proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) for the Treatment of Peanut Allergy

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Abstract

Background—Immunotherapy for peanut allergy may be limited by the risk of adverse reactions.

Objective—To investigate the safety and immunologic effects of a vaccine containing modified peanut proteins.

Methods—This was a Phase 1 trial of EMP-123, a rectally administered suspension of recombinant Ara h 1, Ara h 2 and Ara h 3, modified by amino acid substitutions at major IgE binding epitopes, encapsulated in heat/phenol killed *E. coli.* Five healthy adults were treated with 4 weekly escalating doses after which 10 peanut allergic adults received weekly dose escalations over 10 weeks from 10mcg to 3063mcg, followed by 3 biweekly doses of 3063 mcg.

Results—There were no significant adverse effects in the healthy volunteers. Of the 10 peanut allergic subjects [4 with intermittent asthma, median peanut-IgE 33.3kU$_A$/L (7.2–120.2), median peanutskin prick test wheal 11.3mm (6.5–18)], 4 experienced no symptoms, one had mild rectal symptoms, and the remaining 5 experienced adverse reactions preventing completion of dosing. Two were categorized as mild but the remaining three were more severe, including one moderate reaction and two anaphylactic reactions. Baseline peanut IgE was significantly higher in the 5 reactive subjects (median 82.4 versus 17.2kU$_A$/L, p=0.032), as was baseline anti-Ara h 2 IgE (43.3 versus 8.3, p=0.036). Peanut skin test titration and basophil activation (at a single dilution) were significantly reduced after treatment but no significant changes were detected for total IgE, peanut IgE, or peanut IgG4.

Conclusions—Rectal administration of EMP-123 resulted in frequent adverse reactions, including severe allergic reactions in 20%.

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Statement of Author Contributions:
Drs. Wood and Sicherer contributed to protocol development, conducted the clinical trial, and participated in analyses and writing of the manuscript. Drs. Lindblad and Stablein and Ms. Henning contributed to protocol development, participated in analyses and writing of the manuscript. Drs. Burks, Sampson, and Grishin participated in analyses and writing of the manuscript.

INTRODUCTION

Peanut allergy is increasingly prevalent, potentially fatal, and significantly impacts quality of life (1–4). Current treatment relies on strict dietary avoidance and ready access to injectable epinephrine. Traditional subcutaneous immunotherapy has proven unsafe for peanut allergy (5) and although oral and sublingual immunotherapy have shown some promise for peanut and other food allergens (6–9), these approaches have the potential for significant adverse reactions (10–13).

Given the risks of administering intact food allergen to highly allergic patients, research efforts have focused on the development of methods to render allergens less likely to induce adverse IgE-mediated reactions, while still maintaining – or even enhancing – the potential to induce tolerance (6). These approaches include immunotolerant peptides (14), engineered recombinant proteins (15), plasmid DNA (16–17), and immunostimulatory sequences (18), among others. Another variation on this theme, which was the focus of this trial, utilizes mutated recombinant proteins administered within heat-killed *Escherichia coli*, with the intent of both reducing clinical reactivity and generating maximal tolerogenic immune responses. The potential utility of this vaccine, comprised of heat/phenol killed, E. coli-encapsulated, recombinant Modified Peanut proteins Ara h 1, Ara h 2, and Ara h 3, (EMP-123), is supported by murine studies in which rectal administration of EMP-123 appeared safe and potentially effective (19). This Phase 1 study was conducted to assess the safety of this vaccine, with the goal of advancing to Phase 2 studies to assess its potential efficacy.

Methods

Study Product

EMP-123 (Allertein Therapeutics, LLC) is a rectally administered vaccine consisting of three recombinant modified peanut antigens encapsulated within heat/phenol inactivated *E. coli*. Specifically, three recombinant modified peanut proteins (Ara h 1, Ara h 2, and Ara h 3) were modified by amino acid substitutions to disrupt common IgE binding sites as previously described (20–21). These modified proteins are then separately expressed in *E. coli* strain BLR(DE3), and the *E. coli* are subsequently killed using heat and phenol. The expressed proteins remain encapsulated within the dead *E. coli*. The three resulting whole-cell suspensions (EMP-1, EMP-2, and EMP-3; i.e., three drug substances) are combined approximately 1 (Ara h 1) : 3.2 (Ara h 2) : 2.9 (Ara h 3) based on the intracellular recombinant protein content of the recombinant modified proteins in phosphate buffered saline (PBS), 10% glycerol, 0.5% phenol, and 0.5% hydroxypropyl methylcellulose (HPMC) to form the drug product (EMP-123).

Subject Enrollment and Dosing Schema

The study was conducted in two phases, with an initial cohort of 5 healthy volunteers followed by 10 peanut allergic subjects (Supplemental Figure 1). Subjects were 18 to 50 years of age with no history of severe anaphylaxis. The healthy volunteers had to ingest peanut regularly, have no asthma history, and have negative skin prick tests (SPT, <3 mm wheal) and specific IgE (<0.35 kU_A/L, ImmunoCAP, Phadia, Uppsula, Sweden) to peanut. The study was conducted under an Investigational New Drug application from the FDA, with approval from the NIAID Data Safety Monitoring Board, the investigational review boards of Mount Sinai and Johns Hopkins, and the NIH Recombinant DNA Advisory Committee.

The healthy volunteers received four escalating doses of study product as a rectal suspension on a weekly basis to achieve the maximum study dose, containing 3,063 μg of total modified

peanut protein delivered rectally as a 7 ml suspension (Supplemental Figure 1). Each dose was administered with a 2 hour observation and subjects maintained a home diary to record any symptoms between visits, as well as weekly telephone interviews for 4 weeks after the last dose.

The second phase of the study enrolled 10 peanut allergic subjects who were required to have a convincing clinical history of peanut allergy, with the development of symptoms (e.g., urticaria, flushing, rhinorrhea and sneezing, throat tightness or hoarseness, wheezing, vomiting) within minutes to 2 hours of ingestion. Additionally, a positive peanut skin prick test (SPT >5 mm wheal) and specific IgE (> 5 kU_A/L) were required. Baseline oral food challenges were not done for ethical reasons given that this was a Phase 1 study. Asthmatic subjects could not be more than mild intermittent in severity. These subjects received weekly dose escalations for 10 weeks to the maximum study dose, which was then administered biweekly for 6 weeks (Supplemental Figure 1). If adverse reactions occurred, only a single repeat dose or dose reduction was permitted. Dosing was not continued beyond 13 doses or 16 weeks, and the dose was not escalated beyond the 3,063 μg maximum study dose. Each dose was administered under observation and subjects were monitored for a minimum of 2 hours. Each dosing visit was followed with a telephone interview on the following day and subjects maintained a home diary between visits. After the final dose, weekly telephone calls were conducted for 4 weeks when a final study visit was completed. Telephone interviews were also conducted 1, 2, 3 and 6 months after the last visit.

Study Objectives

The primary objective of this study was to assess the safety of rectally administered EMP-123, first in healthy volunteers and then in 10 peanut allergic subjects. The primary outcome measure was the percentage of subjects who successfully completed dosing with no more than mild symptoms. The highest dose that ≥50% of the allergic subjects tolerated with no more than mild symptoms, and at which no subjects experienced severe symptoms, was the pre-defined maximum dose limit.

Secondary outcome measures included the rate of all serious and overall adverse events, the rate of desensitization (as determined by peanut endpoint SPT titration in peanut allergic subjects pre- and post-treatment), and changes in basophil activation and peanut-specific IgE and IgG4. Additional post-hoc analyses were undertaken to explore IgE binding to the study product pre- and post-treatment.

Laboratory Methods

Serologic Studies—Total IgE and peanut-specific IgE and IgG4 were measured at baseline and week 8 for healthy volunteers and weeks 16 and 20 for peanut allergic subjects, as was anti-Ara h 1, -Ara h 2, and -Ara h 3 IgE. In a post hoc analysis, IgE levels to native peanut, recombinant peanut, and the modified recombinant proteins were measured using an enzyme-linked immunosorbent assay (ELISA). Binding of the peanut-specific IgE to native and recombinant proteins was detected with a horseradish peroxidase–labeled goat anti-human IgE (Sigma-Aldrich) secondary antibody using ABTS substrate (KPL, Inc, Gaithersburg, Md) and absorbance at 405 nm. IgE binding to whole modified proteins were also assessed at baseline and week 20.

Skin Prick Testing—A SPT to peanut extract (Greer Laboratories, Lenoir, NC, USA) was performed in healthy volunteers and peanut allergic subjects at baseline and at week 8 for healthy volunteers and weeks 16 and 20 for peanut-allergic subjects. In addition, end-point titration SPT was performed with the same extract in peanut allergic subjects with four serial ten-fold dilutions of the 1:20 wt/vol peanut extract (1:20, 1:200, 1:2000, and 1:20,000) at

baseline and at week 20, and changes were assessed by measuring the area under the curve (AUC) calculated as the sum of the wheals at each of the 5 serial dilutions.

Basophil activation—Flow cytometry-based assessment of basophil activation / degranulation with and without *in vitro* stimulation was assessed to track changes induced by EMP-123 at baseline, week 16, and week 20 utilizing 0.25 mL of whole blood as previously described.(22) After a 30-minute incubation, cells were fixed and shipped to the central laboratory. Samples were then analyzed by flow cytometry for surface expression of activation markers (CD63, CD203c and CD69).

Statistical Analysis

The sample size for this Phase 1 first-in-humans safety study was selected to be 5 healthy volunteers and 10 peanut allergic subjects, which would provide for initial assessment of safety parameters. If symptomatic response was sufficiently limited, this study group would permit larger subsequent studies for further evaluations of product safety and efficacy. If no peanut allergic subject developed significant dose-limiting allergic reactions, the upper 95% one-sided confidence limit for this event would be 0.26. Exact 2 sample Wilcoxon tests were used to compare baseline antibody levels of reactive and non-reactive subjects.

Results

All 5 healthy subjects tolerated all 4 doses except for diarrhea or loose stools post-dosing occurring in two subjects. No dose adjustments were required and no immunological changes were noted after dosing. The remainder of the results will focus on the allergic subjects.

Baseline demographic and laboratory data for the 10 peanut allergic volunteers are presented in Table 1. There were 6 males and 4 females (median age, 24 years). Four had a history of asthma, the median peanut SPT wheal was 11.3 mm (range 6.5 – 18 mm), and the median peanut IgE level was 33.3 kU_A/l (range 7.2 – 120 kU_A/l).

Four of the 10 subjects completed dosing without any adverse reactions. One subject experienced rectal pruritus at the 3rd and 7th dose but completed all 13 doses and was considered non-reactive. The remaining 5 subjects experienced adverse reactions that prevented them from completing dosing (Table 2). Two of these reactions were categorized as mild but, per protocol, required discontinuation of dosing. The remaining three subjects experienced more severe reactions, including one moderate reaction at dose 12 (flushing, pruritus, throat discomfort, and wheezing), one anaphylactic reaction at dose 9 (nausea, diarrhea, flushing, hives, hoarseness / throat tightness), and one anaphylactic reaction at dose 11 (abdominal pain, diarrhea, congestion, pruritus, shortness of breath, throat tightness / hoarseness). Each of these reactions occurred within one hour of dosing.

Analyses were conducted to investigate clinical or immunologic differences between the reactive and non-reactive subjects, as well as to assess for immunologic changes before and after treatment (Table 3). Baseline peanut IgE levels were significantly higher for the 5 reactive subjects (median 82.4 kU_A/L versus 17.2, p=0.032). Anti-Ara h 2 IgE was also higher in the reactive subjects (43.3 vs. 8.3, P=0.03), while skin test results were similar (p=0.88). There were no significant changes in peanut IgE from baseline to week 20 (median change −1.5 kU_A/L for non-reactive subjects compared to −14.7 kU_A/L for reactive subjects). No baseline differences were found for peanut IgG4, which also did not change significantly from baseline to week 20. However, changes after treatment were detected for peanut endpoint titration SPT, for which the median change in AUC decreased from baseline for all subjects to week 20 (Supplemental Table 1, −10.0 within-subject change;

p=0.02). Basophil activation was assessed at baseline and weeks 16 and 20. The only significant change was an increase from baseline in %CD63+ at the 0.01 μg/ml concentration (p=0.05) (Supplemental Figure 2). There were no other trends over time or differences between the reactive and non-reactive subjects, including measures of peanut specific IgA and IgA2.

Additional post-hoc analyses were performed to further assess differences between reactive and non-reactive subjects (Table 3), including standard peanut specific IgE, anti-Ara h 1, -Ara h 2, and -Ara h 3, total IgE, and IgE against wild type recombinant peanut, native peanut, and the modified recombinant proteins used in treatment. Aside from the differences in anti-peanut and anti-Ara h 2 differences noted above, the only other significant differences were detected for recombinant Ara h 2 and Ara h 3. While there were trends toward differences in IgE to the modified recombinant proteins, these were not significant.

Discussion

EMP-123 was developed as a vaccine to induce tolerance to the dominant peanut proteins, Ara h 1, 2 and 3, with the rationale that disruption of sequential IgE binding epitopes on wild type peanut proteins may reduce the ability of these proteins to induce adverse allergic responses. Additional rationale included delivery of the modified peanut proteins within an intact cellular delivery system (i.e., *E. coli*) to further reduce the potential for allergic reactions, and the use of bacteria themselves to enhance tolerance by providing antigen-presenting cells activation factors to promote induction of Th1 immunity, and potentially suppress antigen-specific Th2 responses. Finally, it was hypothesized that the rectal route of administration might further enhance the development of tolerance given the rich immunologic environment of the lower colon. Theoretically, therefore, this approach could enhance both safety and efficacy compared to immunotherapy using intact peanut proteins, and might also shorten the course of therapy needed to induce tolerance if larger antigen doses could be safely administered.

Unfortunately, however, while the product appeared safe in the healthy volunteers, adverse reactions were common in the peanut allergic subjects. Fifty percent of those participants were unable to complete the dosing regimen. While the study stopping rules were very stringent for this Phase 1 study, 3 of the 10 peanut allergic participants did experience significant allergic reactions to the product. This was more likely to occur in those with higher baseline peanut IgE levels, although these levels were not exceptionally high relative to those encountered in routine clinical practice. Co-existent asthma did not appear to be a factor, but this conclusion is limited by exclusion of patients with persistent asthma.

Given the marked heterogeneity of epitope recognition by peanut allergic patients (23), it would not be surprising that individual patients may have serum IgE that recognizes as yet unidentified epitopes, or that critical amino acids were not modified in the current EMP-123 product. Thus, while reduced IgE binding was anticipated for most patients (21), it was not expected that the modified allergens in EMP-123 would have reduced IgE binding in all subjects. However, the frequency and intensity of the reactions that occurred in this small number of subjects was unexpected. Surprisingly, when analyzing IgE levels to the modified proteins, differences were not detected between the reactive and non-reactive subjects. However, our failure to detect these differences may simply reflect the small sample size, as trends were noted with regard to IgE levels to the modified proteins, or that subjects were reacting to something else in the vaccine product.

It is possible that these reactions could have been minimized or avoided if a more conservative approach to dosing had been undertaken, potentially including slower

escalation and daily dosing. For example, in recent oral and sublingual immunotherapy trials, it is typical to administer doses on a daily basis and to escalate dosing over a period of 4–6 months (7, 24–25). We had anticipated that a more rapid escalation and weekly dosing would be tolerated using this modified product, and although we do not know that a different approach to dosing would be beneficial, it is certainly possible that it would be. We also do not know if the rectal route of delivery added to the risk of adverse reactions due to its highly absorptive nature.

There was no effort to assess efficacy in this Phase 1 trial, but we included several measures to evaluate immunologic changes with treatment. No changes in peanut IgE or IgG4 were detected. There was, however, a significant change in skin test responses measured by end point titration, as well as basophil reactivity at the 0.01 ug concentration. Although it is impossible to know whether these changes have any clinical significance, the SPT results are similar to those seen in a study of peanut SLIT where modest changes in food challenge threshold were seen after treatment (26).

In conclusion, as tested, this vaccine led to frequent and sometimes severe allergic reactions, in spite of the clear scientific rationale and pre-clinical studies behind its development. Future studies with this product, if any, will require alterations in the dosing scheme and / or route of delivery. The overall approach, however, of using modified allergens for immunotherapy, still holds great interest for all the reasons underlying the development of this product. While other immunotherapeutic methods currently under investigation certainly hold promise approaches that could enhance both safety and efficacy are still highly desirable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of Interest:

R. A. Wood has consultant arrangements with the Asthma and Allergy Foundation of America, is employed by Johns Hopkins University, has received research funding from the NIH and the Food Allergy Initiative, and received royalties from UpToDate.

S. H. Sicherer has received grants from the NIH/NIAID and has consultant arrangements with the Food Allergy Initiative and Novartis, and has received royalties from UpToDate.

A.W. Burks has board memberships with the American Academy of Allergy, Asthma & Immunology, the NIH Hypersensitivity, Autoimmune, and Immune-mediated Diseases study section, the US Food and Drug Administration, and the Journal of Allergy and Clinical Immunology; is on advisory boards for the Food Allergy & Anaphylaxis Network, ActoGeniX, and Exploramed Development; has consultant arrangements with Merck, Novartis Pharma AG, the Dannon Company, McNeill Nutritionals, and Schering-Plough; is employed by UNC Children's Hospital and Duke University; has received grants from the NIH; has grants pending from the

Department of Defense and the Wallace Research Foundation; receives payment for lectures from Myland Specialty; receives royalties from UpToDate; receives payment for development of educational presentations from Current Views; has stock/stock options with Allertein Therapeutics, LLC, Mastcell Pharmaceuticals, and Dow AgroSciences.

Alexander Grishin is a paid consultant to Allertein Therapeutics, LLC and receives research funding from the NIH.

A. K. Henning has received grants from the NIH.

R. Lindblad has received grants from the NIH/NIAID.

D. Stablein has received grants from the NIH.

H. A. Sampson serves as a paid consultant to Allertein Therapeutics, LLC and the Food Allergy Initiative, has a financial interest in Allertein Therapeutics, LLC and Herb Spring, LLC, receives royalties from UpToDate and Elsevier, and receive research funding from the NIH and the Food Allergy Initiative.

Due to their involvement with Allertein Therapeutics, LLC, Drs. Burks, Grishin and Sampson were not permitted to have any role in the development or conduct of the clinical trial.

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Table 1

Baseline demographic and laboratory data (minimum-maximum (median)) for the 10 peanut allergic subjects

Gender	**6 male / 4 female**
Age (yrs,median)	19 – 35 (24)
Other food allergy	10
Tree nuts	7
Other	3
Asthma (mild intermittent only)	4
Peanut skin test wheal (mm)	6.5 – 18 (11.3)
Peanut IgE (kU_A/L)	7.2 – 120.2 (33.3)
Peanut IgE Ara h 1 (kU_A/L)	4.9 – 63.3 (21.6)
Peanut IgE Ara h 2 (kU_A/L)	2.9 – 119.6 (18.9)
Peanut IgE Ara h 3 (kU_A/L)	0 – 14.2 (2.1)
Peanut IgG4 (mg_A/L)	0.5 – 17.0 (4.7)
Peanut IgG4 Ara h 1 (mg_A/L)	0.09 – 0.72 (0.26)
Peanut IgG4 Ara h 2 (mg_A/L)	0.14 – 1.05 (0.24)
Peanut IgG4 Ara h 3 (mg_A/L)	0.04 – 0.77 (0.08)

Table 2

Peanut Allergic Subjects Experiencing Allergic Reactions to the Study Drug

Subject Number	Baseline Peanut-IgE	Asthma	Last Dose (mcg)	Final Status
M1	120.2	no	3063	Moderate reaction at dose 12 (flushing, pruritus, throat discomfort, and wheezing); refused epinephrine); discontinued from study per protocol.
J1	82.4	no	3063	Mild reaction (severe crampy abdominal pain) at dose 4; dose repeated and re-escalated but had same reaction at dose 12 - discontinued study drug per protocol.
M7	90.3	yes	1750	Mild abdominal discomfort, flushing, and diarrhea after dose 9, repeated dose and experienced mild abdominal discomfort - discontinued study drug per protocol.
J2	28.3	no	875*	Anaphylaxis at dose 9 (nausea, diarrhea, flushing, hives, hoarseness / throat tightness); given epinephrine with resolution; discontinued from study per protocol.
J3	38.2	no	3063	Anaphylaxis at dose 11 (abdominal pain, diarrhea, congestion, pruritus, shortness of breath, throat tightness / hoarseness); treated with epinephrine with resolution; discontinued from study per protocol.
M2	7.2	no	3063	None
M3	73.6	yes	3063	None
M4	11.2	yes	3063	None
M5	17.2	no	3063	None
M6	26.2	yes	3063	Mild rectal pruritus at 40 and 437.5 mcg doses

* Subject J2 had one earlier dose repeated and was therefore on lower dose at dose 9 than subject M7

Table 3

Results of serologic studies comparing peanut allergic subjects with or without treatment related reactions.

	Not Reactive		Reactive		
Variable	**Baseline (N=5)**	**Week 20 (N=5)**	**Baseline (N=5)**	**Week 20 (N=4)**	**P Value**
ImmunoCAP IgE (kU_A/L) (Median Values)					
Peanut	17.17	19.82	82.35	56.62	0.03
Ara h 1	10.28	…	25.48	…	0.15
Ara h 2	8.27	…	43.26	…	0.03
Ara h 3	1.75	…	2.87	…	0.42
Total IgE	900.11	…	381.97	…	0.31
ImmunoCAP IgG4 (mg_A/L) (Median Values)					
Peanut	2.23	2.13	5.20	1.60	0.22
Ara h 1	0.15	…	0.34	…	0.25
Ara h 2	0.19	…	0.47	…	0.03
Ara h 3	0.06	…	0.08	…	0.11
ELISA (OD@405nm) (Mean Values)					
Native Ara h 1	0.80	0.62	1.59	1.80	0.056
Native Ara h 2	0.48	0.35	1.44	1.51	0.03
Wild type Ara h 1	0.16	0.14	0.18	0.18	0.34
Wild type Ara h 2	0.31	0.25	0.90	0.95	0.03
Wild type Ara h 3	0.08	0.08	0.34	0.33	0.008
Modified Ara h 1	0.12	0.13	0.20	0.21	0.15
Modified Ara h 2	0.09	0.11	0.19	0.16	0.15
Modified Ara h 3	0.10	0.10	0.37	0.35	0.056

Notes:
- Wild type refers to recombinant, unmodified proteins.
- P-value reflects comparison of not reactive to reactive at baseline using the exact Wilcoxon test.
- Results for wild type, native, and modified proteins represent OD assessments at 1:100 dilutions
- Ara h 1, Ara h 2, and Ara h 3 were analyzed with ImmunoCAP at baseline only and not at Week 20.