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Safety, clinical and immunologic efficacy of a Chinese herbal medicine (FAHF-2) for food allergy

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Abstract

Background—FAHF-2 is a 9-herb formula based on Traditional Chinese Medicine that blocks peanut anaphylaxis in a murine model. In Phase I studies, FAHF-2 was found to be safe, and well tolerated.

Objective—To evaluate the safety and effectiveness of FAHF-2 as a treatment for food allergy.

Methods—In this double-blind, randomized, placebo-controlled study, 68 subjects, 12-45 years of age, with allergies to peanut, tree nut, sesame, fish, and/or shellfish, confirmed by baseline double-blind, placebo controlled food challenge (DBPCFC), received FAHF-2 (n=46) or placebo (n=22). After 6 months of therapy, subjects underwent DBPCFC. For those who demonstrated increases in eliciting dose, a repeat DBPCFC was performed 3 months after stopping therapy.

Results—Treatment was well-tolerated with no serious adverse events. By intent-to-treat analysis, the placebo group had a higher eliciting dose and cumulative dose (p=0.05) at the end of treatment DBPCFC. There was no difference in the requirement for epinephrine to treat reactions (p=0.55). There were no significant differences in allergen-specific IgE and IgG_4, cytokine

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production by PBMCs or basophil activation between active and placebo groups. *In vitro* immunological studies performed on subject baseline PBMCs incubated with FAHF-2 and food allergen produced significantly less IL-5, greater IL-10 and increased numbers of Tregs than untreated cells. Notably, 44% of subjects had poor drug adherence for at least one-third of the study period.

Conclusion—FAHF-2 is a safe herbal medication for food allergic individuals and shows favorable in vitro immunomodulatory effects; however, efficacy for improving tolerance to food allergens is not demonstrated at the dose and duration used.

Keywords

food allergy; FAHF-2; Chinese herbal therapy; peanut allergy

Introduction

Food allergy affects as many as 8% of young children and 5% of adults.[1] Peanut allergy is the leading cause of food-induced anaphylaxis in the U.S.[2] The standard of care for food allergy management entails strict avoidance and immediate access to rescue medications,[3] and currently, there is no effective therapy or cure.

Traditional Chinese Medicine (TCM) has been used in China to treat various diseases for thousands of years, particularly in the form of herbal formulas. Recently, TCM has been attracting interest in Western countries as a source of alternative or complementary therapy for a variety of diseases, including allergies and asthma.[4-8] FAHF-2 is the first botanical investigational new drug (IND) approved for clinical studies for food allergy by the US Food and Drug Administration (FDA). FAHF-2 is a 9-herb formula based on the classical Chinese herbal formula *Wu Mei Wan*.[9] Murine model studies as well as phase I acute and extended trials of FAHF-2 in patients with peanut, tree nut, fish and/or shellfish allergy demonstrated that this formula is safe and well tolerated; and as seen in a murine model of peanut allergy, it has beneficial immunoregulatory effects in vitro.[10-15]

Therefore, the aim of this study was to examine the safety and efficacy of FAHF-2 for the treatment of food allergy in a multi-center, randomized, double-blind, placebo-controlled clinical trial. Based on previously published data, we proposed that the therapeutic effect of FAHF-2 on food allergy is due to prevention of IgE triggered mast cell/basophil activation and suppression of Th2 cell cytokine production. Thus, a secondary aim was to examine the immunomodulatory effects of FAHF-2 in humans.

Methods

Study participants

Food allergic individuals ages 12-45 years of age with a convincing history of allergy to peanut, tree nut (almond, cashew, hazelnut, pecan, pistachio, walnut), sesame, fish (cod, tuna, salmon, catfish) or shellfish (crab, lobster, shrimp) as documented by a positive skin test (mean wheal diameter ≥5 mm greater than the mean of saline control) and/or food allergen-specific IgE level (IgE ≥ 0.7 kU_A/L) and positive double-blind, placebo controlled

oral food challenge (DBPCFC) (total 2 grams of protein) were eligible for the study. Only one food allergen was chosen to be studied during the trial for each participant. Females of childbearing potential were included but had to be sexually inactive or using effective birth control measures.

Subjects with a history of life-threatening anaphylaxis (involving hypotension or requiring mechanical ventilation) were excluded. Additional exclusion criteria included history of systemic disease that in the investigator's opinion would preclude the subject from participating in this study (e.g. autoimmune disease, neoplasms, HIV or hepatitis virus infection, bleeding disorders/diatheses, history of breast and/or ovarian cancer); abnormal hepatic, bone marrow or renal function; clinically significant abnormal electrocardiogram; current uncontrolled moderate to severe asthma with FEV1 <80% predicted; drug or alcohol abuse; pregnancy or lactation; use of omalizumab; and participation in another research protocol within the previous 30 days.

This study was approved by the Institutional Review Boards at each clinical site. Subjects were recruited from 3 U.S. sites (Icahn School of Medicine at Mount Sinai, New York, NY; Arkansas Children's Hospital, Little Rock, AR; Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, IL).

The study was conducted under an investigational new drug application to the Food and Drug Administration (FDA) (77,468) and was monitored by an independent data and safety monitoring board. Written informed consent was obtained prior to enrollment; assent was obtained for children 12-17 years of age.

Study Design

This was a randomized, double-blind, placebo-controlled trial. Subjects were randomized using a centralized computer generated algorithm to receive FAHF-2 or placebo (2:1), 10 tablets three times a day for 6 months. The primary endpoint was the percentage of subjects who could consume, without dose-limiting symptoms, 2 grams of protein or a greater than a 4-fold increase in the allergen dose to induce a positive DBPCFC after therapy compared to baseline. Secondary outcomes assessed the rate of adverse events as well as immunologic parameters.

The screening evaluation entailed a medical history, physical examination, skin prick testing, food-specific IgE testing, pulmonary function test, electrocardiogram, urinalysis, and routine laboratory blood tests (complete blood count, serum chemistries, renal function, liver function tests, and pregnancy test for female participants). Subjects underwent a baseline DBPCFC (up to 2 grams protein).

Subjects continued food allergen avoidance and refrained from other herbal medication use. Subjects were contacted by telephone weekly for the first 4 weeks, then every 2 weeks to assess medication adherence and potential adverse events (AEs). Interval history, physical examination, laboratory tests, and symptom diary were evaluated every 8 weeks at study visits.

Post-therapy, a 5 gram protein DBPCFC was performed. Subjects who demonstrated an improvement in challenge eliciting dose (amount of food allergen that could be consumed without dose-limiting symptoms) as defined for the primary endpoint returned for a DBPCFC 3 months off therapy to assess for sustained effect.

Study Medication

FAHF-2 tablets (0.5g/tablet) were produced by Xiyuan Chinese Medicine Research and Pharmaceutical Manufacturer, China. The quality of raw herbs, manufacturing process and quality control of the final FAHF-2 product was established according to FDA guidance under the botanical drug title (Chemical, Manufacturing, and Control Data [21 CFR 312.23(a) (7)]) as published previously.[14]

Placebo tablets were identical in appearance, but contained corn starch (0.55 g/tablet). These tablets were manufactured by the same company as FAHF-2.

Study procedures

Skin prick testing—Endpoint titrated skin prick tests (SPTs) with serial 10-fold dilutions were performed at baseline and after the treatment phase. The standard extracts (1:20 wt/vol) of stock peanut, tree nuts (almond, cashew, hazelnut, pecan, pistachio, walnut), sesame, fish (cod, tuna, salmon, catfish) or shellfish (crab, lobster, shrimp) (Greer Laboratories; Lenoir, NC) were used. Negative controls (phenol-saline solution) and positive controls (1 mg/ml histamine base) were also included. SPTs were performed by pricking with a GreerPick (Greer Laboratories) through a drop of extract placed on the volar aspect of the forearm. The mean of the largest orthogonal diameters of the wheal was recorded. A wheal diameter at least 3 mm greater than the negative control was considered a positive response.

DBPCFC—At baseline and post-therapy, subjects underwent DBPCFC which entailed gradually feeding increasing amounts of the food allergen to a maximum of 2 grams (baseline DBPCFC) or 5 grams (post-therapy DBPCFCs) protein at 10-15 minute intervals under supervision. All sites used the same procedure. The doses were distributed in the following manner: 2 grams (1, 5, 15, 50, 75, 100, 250, 500, and 1000 mg) and 5 grams (1, 5, 15, 50, 75, 100, 250, 500, 1000, 1250, and 1750 mg).

A DBPCFC was considered positive when a subject developed cutaneous (urticaria, angioedema, and/or flushing), gastrointestinal (abdominal cramping, vomiting, and/or diarrhea), respiratory (persistent nasal congestion, persistent rhinorrhea, persistent sneezing, tightness in the throat, dysphonia, dyspnea, and/or wheezing), neurologic (change in activity level and/or confusion) and/or cardiovascular (dizziness, loss of consciousness, and/or hypotension) symptoms. DBPCFCs were also stopped if persistent subjective symptoms were reported.

Immunological Studies

Allergen-specific IgE and IgG_4 measurements—At each study visit, allergen-specific IgE (sIgE) to the study food allergen was measured using ImmunoCAP® (Thermo

Fisher Scientific, Waltham, MA). At baseline and post-therapy, allergen-specific IgG_4 ($sIgG_4$) was measured using ImmunoCAP®.

Cytokine profiles and basophil activation—At baseline and post-therapy, serum cytokine profiles and basophil activation (*ex vivo* studies) were determined in both active and placebo subjects (Repository Methods E1 and E2).[19,20]

In order to assess the response to direct exposure to FAHF-2 and predict clinical outcome, *in vitro* studies were performed. PBMCs obtained from subjects at baseline (pre-treatment) were incubated with FAHF-2 plus food allergen *in vitro*, and cytokine profiles and T regulatory cell numbers were determined to correlate cellular responses to FAHF-2 with clinical outcome. (Repository Methods E3).[14,16]

Safety Monitoring

Subjects were monitored for potential AEs based on criteria approved by the FDA that were adapted from the World Health Organization (WHO) Recommendations for Grading of Acute and Subacute Toxicity.[14]

Statistical Analysis

A sample size of 68 subjects (allowing for a 20% drop out rate) would yield 36 active and 18 placebo evaluable patients, providing a power of 83% to detect a difference between an estimated 60% success rate in meeting the primary endpoint in the active group and 20% success rate in the placebo group, using a two-tailed Chi-Square test of equal proportions at a 5% level of significance. The predicted success rate in the placebo group was based on a prior study where a greater than 4-fold increase in threshold dose was reported in 20% of the placebo group.[17]

Comparison of categorical data was performed with Fisher's exact test with a 2-tailed p value, while comparison of continuous data was performed with a t-test. Analysis of Covariance (ANCOVA) models were used to assess whether the change from baseline to post-therapy in various outcome variables (sIgE, $sIgG_4$, IL-5, IL-10, IFN- γ, basophil activation, SPTs) differed between treatment groups.[18] The change in the outcome was the dependent variable and the following were independent variables: treatment group, baseline value, treatment-by-baseline interaction, adherence to treatment, and clinical center. In addition, random-effects models were used to evaluate the longitudinal responses for each of the outcome variables.[19] These models included responses at 2, 4, 6, and 9 months after treatment initiation, and in addition to the independent variables listed in the models above, time was a predictor variable as well as the interaction between time and treatment group. This interaction term assessed whether time trends in response differed between treatment groups.

Statistical analyses for the *in vitro* immunologic studies were performed using a left censored log-normal repeated measure tobit model and a mixed model with a random intercept on natural log transformed Treg percentages.

All analyses were conducted using SAS v. 9.2.

Results

Subject characteristics

Sixty-eight subjects were randomized; one withdrew within the first 4 weeks and was replaced per protocol (Figure 1). The median age of the subjects was 16 years (range 12-44 years), and 61.7% were males (Table 1). Peanut was the study allergen for 73.5%. Twenty-six (38.2%) had a history of food-induced anaphylaxis. Subjects were highly atopic: 88.2% had multiple food allergies 73.5% had asthma, 70.6% had allergic rhinitis, and 51.5% had atopic dermatitis.

At the baseline DBPCFC, there were no differences in eliciting dose, cumulative dose or requirement for epinephrine between treatment groups (Table 1). Furthermore, eliciting dose did not vary by food.

Clinical outcomes

Fifty-nine subjects (86.8%) completed 6 months of treatment. One did not return for the post-therapy DBPCFC, leaving 58 evaluable subjects. There was no significant difference between active and placebo groups in terms of completing the study (37 active, 21 placebo, p=0.09). Based on the primary endpoint parameters set forth in the protocol, significantly more placebo-treated subjects met the primary endpoint of having improvements in consumed allergen dose at the post-therapy DBPCFC as compared to those on treatment (45.5% success in the placebo group vs 17.4% success in the active group, p=0.01). Using intent-to-treat (ITT) analysis, the placebo group had a trend for higher eliciting dose and cumulative dose at the post-therapy DBPCFC (p=0.07) (Table 1, Figure 2). There was no difference in the requirement for epinephrine to treat reactions (p=0.55) (Table 1). Adjusting for adherence also did not alter these results.

Subset analyses of Caucasian race (87% of subjects) and peanut allergy (74%) showed no difference between treatment groups for the primary endpoint of improvement in consumed allergen dose at the post-therapy DBPCFC (16.7% success in the active group vs. 33.3% in the placebo group p=0.17; 14.7% success in the active group vs. 37.5% in the placebo group, p=0.14, respectively). No differences in eliciting or cumulative doses at baseline and post-therapy DBPCFC were observed in these subgroups.

For those meeting the primary endpoint, a repeat 5 gram DBPCFC was performed 3 months off treatment. Eight from the active group and 10 from the placebo group met this criterion. Two from the placebo group declined to participate in this DBPCFC. There was no significant difference between groups for persistence of effect (5 of 8 active vs 3 of 10 placebo; ITT p=0.34).

Additional *post hoc analyses* were performed using more stringent criteria as used in the NIH-funded Consortium of Food Allergy Research (CoFAR) study protocols including:1) must tolerate at least 500 mg of food protein if subject tolerated 0-25 mg at baseline food challenge; 2) must tolerate at least a 10-fold increase in food protein if tolerated 75-250 mg at baseline food challenge; 3) must tolerate 5 gm or more of food protein if tolerated >500 mg at baseline). Using these criteria, there was no difference between active and placebo

groups in achieving improved tolerance (2% success in the active group vs 13.6% success in the placebo group, p=0.08). In addition, there was no difference in persistence of effect.

Adherence to therapy

Adherence was assessed based on the number of tablets taken (calculated by number of tablets returned subtracted from the number dispensed) in relation to the expected number taken during the study time frame (study visits occurred every 2 months). Subjects were considered adherent if medication completion was ≥ 80%. Non-adherence increased over the course of the study; 44% of subjects had poor adherence for at least one-third of the study period (Table 2). There was no difference in adherence between active and placebo groups (p=0.17).

Clinical Adverse Events

A total of 387 adverse events (AEs) were reported; none were severe. There was no difference in the number of AEs reported per subject between active and placebo groups (Table 3). Gastrointestinal complaints were most common. There was no difference between groups in terms of the proportion of gastrointestinal complaints that were associated with study medication dosing (active: 16/61, placebo: 6/22, p=0.80)

Nine subjects withdrew from the study (Figure 1): 4 cited difficulties with compliance, 4 had persistent abdominal complaints, 1 developed a new rash for which the subject wanted to pursue Chinese herbal treatment prescribed by the subject's acupuncturist. All subjects who withdrew due to persistent abdominal complaints were on active treatment. One of these subjects was an early drop-out (within the first 4 weeks) and was replaced as per protocol. For this subject and one other, symptoms resolved within 2 weeks of discontinuing study medication; no other interventions were required. For the remaining 2 subjects, one was subsequently diagnosed with non-celiac gluten sensitivity that responded well to a gluten-free diet; the other was diagnosed with a peptic ulcer and symptoms resolved after starting lansoprazole. The subject with new rash had been randomized to active treatment. Two subjects in the active group were lost to follow-up; 1 completed treatment, but did not finish the DBPCFC due to scheduling conflicts.

Immunological test results

There were no differences between treatment groups at baseline and at the end of therapy for all laboratory parameters measured. Pulmonary function studies and electrocardiogram findings did not change following treatment.

While a significant decrease in basophil activation at 200ng/mL in the active group from baseline to the end of the study (p=0.004) was observed, there was no significant difference in the change for this parameter when comparing the active and placebo groups (p=0.1). There were no significant changes in sIgE, $sIgG_4$, sIgE/$sIgG_4$ ratio, and IL-10 and IFN- γ levels between treatment groups (Table 4). A significant increase in IL-5 was observed in the active group, but no change was seen in the placebo group. In addition, sIgE levels did not change over time in either treatment group, and there was no difference between slopes (p=0.9859). Adjusting for adherence did not change these results.

No difference in baseline median SPT was detected between groups (Table 1). Endpoint titration SPTs before and after treatment comparisons found a greater median change for the area under the SPT endpoint titration curve for the placebo group compared to the active group (p=0.03).

In Vitro Immunomodulatory Effects of FAHF-2 on PBMCs obtained at baseline

In order to assess the response to direct exposure to FAHF-2 and predict clinical outcome, *in vitro* studies were performed with PBMCs obtained from subjects at baseline. In the initial experiments, we tested 2 doses. As shown in Figure 3A. Ag+250 μg/mL of FAHF-2 showed significantly lower levels of IL-5 and higher levels of IL-10 compared to cultures with allergen alone (n=12). There was no difference in cytokine levels between PBMCs cultured with Ag alone and Ag+125 μg/mL FAHF-2. We then determined the effects of 250 μg/mL of FAHF-2 and found a reduction in IL-5 and increase in IL-10 (Allergen+FAHF-2 vs. Allergen, $p<0.05$, n=53, Figure 3B) Significantly increased number of $CD4^{+}CD25^{+}$ $FoxP3^{+}$ T regulatory cells was exhibited in the FAHF-2 treated condition (FAHF-2+Allergen vs. Allergen, $p<0.05$, n=10, Figure 4).

Discussion

FAHF-2 is a 9-herb formula that is highly safe and effective in murine models of peanut and multiple food allergies.[10-13] Based on the favorable results of the acute and extended phase I studies,[14,15] we performed a multi-center, randomized, double-blind, placebo-controlled phase II clinical trial to assess safety and efficacy in food allergic individuals.

The results of this study did not demonstrate efficacy of FAHF-2 using a dose of 10 tablets three times a day for 6 months. There was also no significant difference over time within groups or between groups for the other immunologic parameters examined.

Our results provide further support of the safety of this herbal medication, with no differences observed between groups in terms of adverse events, routine laboratory parameters, pulmonary function studies or electrocardiograms. Although no differences in gastrointestinal side effects were reported between groups, 4 subjects receiving active treatment withdrew due to gastrointestinal complaints, suggesting that the number of tablets and/or herbal medication may adversely affect certain individuals. However, of those who were able to complete 6 months of therapy, adherence to study medication was no different between treatment groups.

A significant limitation to this study was the unequal rates of withdrawal (21% in the active group vs. 5% of the placebo group), which can limit assessments of safety and efficacy. Several additional limitations may have affected our ability to detect efficacy. First, the dose used was based on an extrapolation from the effective murine dose using body surface area while also considering the tablet burden that can negatively impact adherence. In this trial, 80% of the full murine dose was chosen, which was 10 tablets three times a day. This high tablet load posed a significant burden on subjects, contributing to drop-out as well as low adherence. Nearly half of the subjects had <80% medication adherence for at least 2 months of the 6 month study and a third were non-adherent during 4-6 months just before the post-

therapy DBPCFC. Thus, suboptimal dosing may contribute to the lack of efficacy seen in this clinical trial. Second, the treatment duration was suboptimal. To achieve a comparable duration to the 7 week treatment in the mouse, 2-3 years of therapy would be required in humans. Data from oral immunotherapy studies also indicate that longer treatment durations are likely to be more effective for well-established food allergy.[20,21]

Animal studies also suggest that concurrent allergen exposure may be necessary for efficacy of FAHF-2. In the murine experiments, the mice were exposed to allergen monthly throughout the study.[12] In this clinical trial, subjects were instructed to maintain strict allergen avoidance, and thus, did not receive concurrent allergen exposure.

Consistent with previous findings,[14] PBMCs cultured with FAHF-2 switched from antigen-induced Th2 to Th1/Treg predominant responses. A higher number of CD4+CD25+Foxp3+ Treg cells were present in FAHF-2+antigen cultures than in cultures with allergen alone. The discrepancy between *in vitro* and *ex vivo* results may be due to direct exposure to sufficient amounts of active compounds *in vitro* that was not replicated *in vivo* under the current clinical study conditions, suggesting that optimizing the treatment dose and more effectively ensuring compliance will be necessary to achieve clinical efficacy.

Results from published food therapy studies indicate that additional factors may influence the ability to modulate the immune system toward tolerance. Oral immunotherapy (OIT) and sublingual immunotherapy (SLIT) studies suggest that an older age at the start of treatment may result in more difficulty achieving desensitization, as success rates tended to be higher in studies that included primarily younger children.[22-24] Furthermore, in 2 studies that included children with a history of anaphylaxis, lower success rates for desensitization were observed in comparison to studies that excluded those with a history of anaphylaxis.[25,26] Subjects in our study were older, with a median age of 16 years, and over a third had a history of anaphylaxis, thus our study population may need optimized doses and prolonged treatment to impact their established allergy.

While the results of this study do not demonstrate clinical efficacy at this dose and duration, several lessons can be learned, in particular, the importance of selecting clinically meaningful endpoint criteria and trial design. Clinically relevant endpoints are necessary as small improvements in cumulative dose in a positive DBPCFC do not provide sufficient protection in case of a true accidental exposure. Using our original endpoint parameters, significantly more placebo-treated subjects experienced improvements in tolerance after 6 months. However, post-hoc analyses using the criteria from the CoFAR group [24] showed no difference between treatment groups. This is due to several subjects being categorized as improved based on small incremental increases in their dose consumed without symptoms during the DBPCFC using the original endpoint parameters, which are likely to be clinically irrelevant. This also suggests that eliciting and cumulative dose may vary over time without treatment.

The need for well-designed, placebo-controlled studies is supported by our observation of clinical improvements in several subjects who received placebo treatment over the relatively

short period of this study. Spontaneous tolerance has also been reported in adolescents in a peanut SLIT study.[24] In recent peanut OIT studies, the improvement rate in placebo groups varied between 0-15%.[27-29] Spontaneous tolerance to tree nuts and fish/shellfish are generally reported to be 9% and 1-2%, respectively.[3] In this study, the improvement rate in the placebo group is higher than previously reported. The reason for this is unknown, requiring further investigation, but study design is known to influence clinical trial success as well. Higher placebo responses have been observed in studies where the chances of receiving active treatment exceed 50% because there is a high expectation of improvement, [30,31] thus leading to poor discrimination between treatment groups. This study randomized subjects 2:1 active to placebo (67% chance of receiving active treatment) in part because of the observation that prospective participants in other food therapy trials with 1:1 randomization schemes were declining participation due to a high likelihood of receiving placebo treatment. In retrospect, this study design may have contributed to our observation that more placebo subjects experienced an increase in cumulative tolerated dose at the post-therapy DBPCFC.

In summary, this study demonstrates that FAHF-2 is a safe herbal medication for food allergic individuals; however, efficacy was not demonstrated at the dose and duration used. Future studies will optimize doses and employ longer treatment durations using the refined formula that has recently been developed which will require fewer tablets.[32] This will also facilitate improved adherence. Improved study design as well as combination therapy to provide concurrent allergen exposure, as in OIT, may enhance our ability to demonstrate efficacy of this herbal product for food allergy.

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Abbreviations

FAHF-2	Food Allergy Herbal Formula-2
PN	Peanut
TN	Tree nuts
DBPCFC	double-blind, placebo-controlled oral food challenge
sIgE	specific IgE

Key Messages

- FAHF-2 is a safe herbal medication for food allergic individuals.
- Efficacy for food allergy is not demonstrated at the dose and duration used. This may be due to study design and adherence problems.

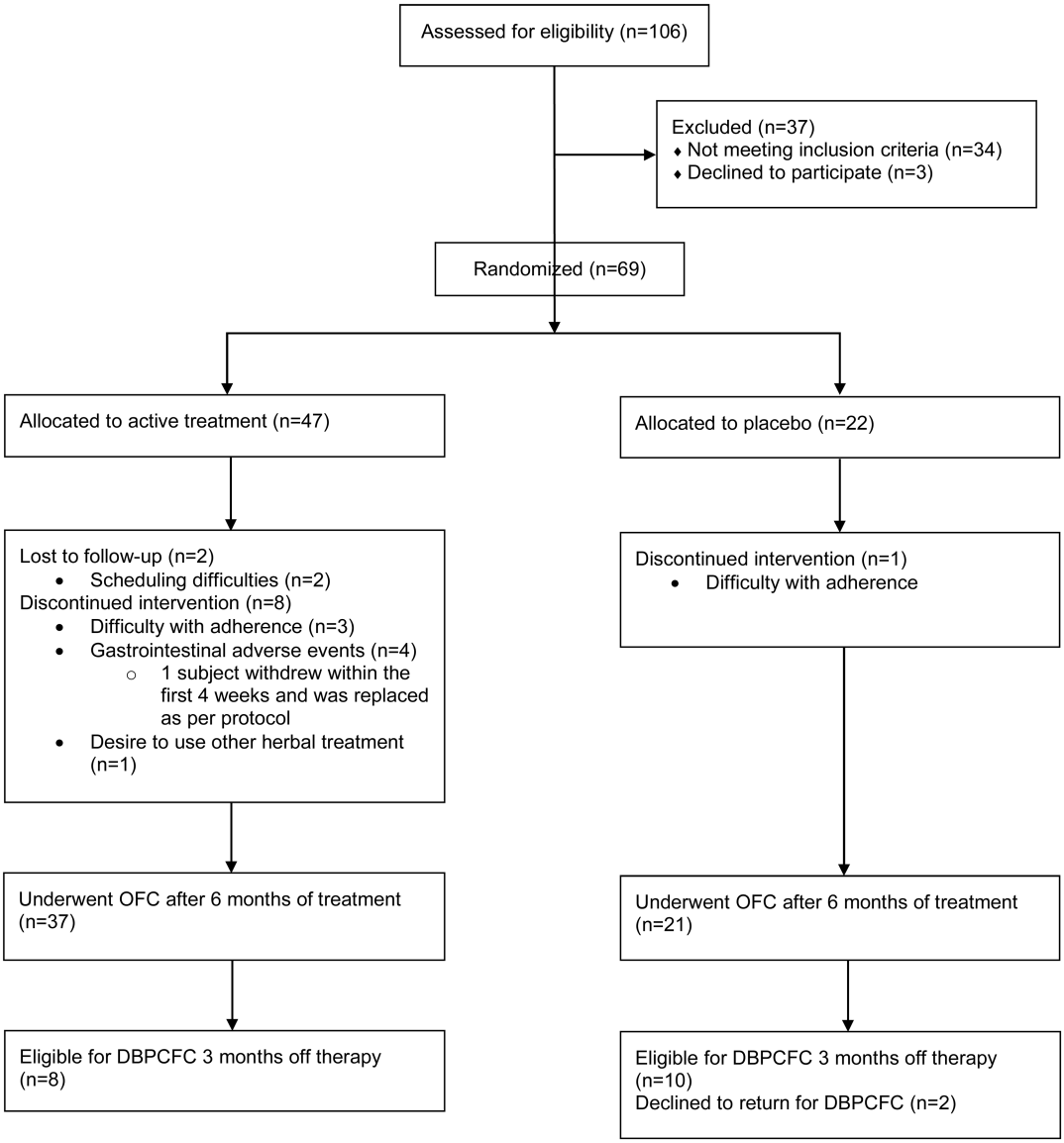

Figure 1.

Consort Flow Chart for this randomized, double-blind, placebo-controlled trial. After screening and baseline 2 gram protein DBPCFC, subjects were randomized to receive FAHF-2 or placebo (2:1), 10 tablets three times a day for 6 months. After 6 months of treatment, a 5 gram protein DBPCFC was performed to assess for effect of the study medication. Subjects who demonstrated an improvement in tolerance (amount of food allergen that could be consumed without dose-limiting symptoms) as defined for the primary endpoint returned for a DBPCFC 3 months off therapy to assess for sustained effect.

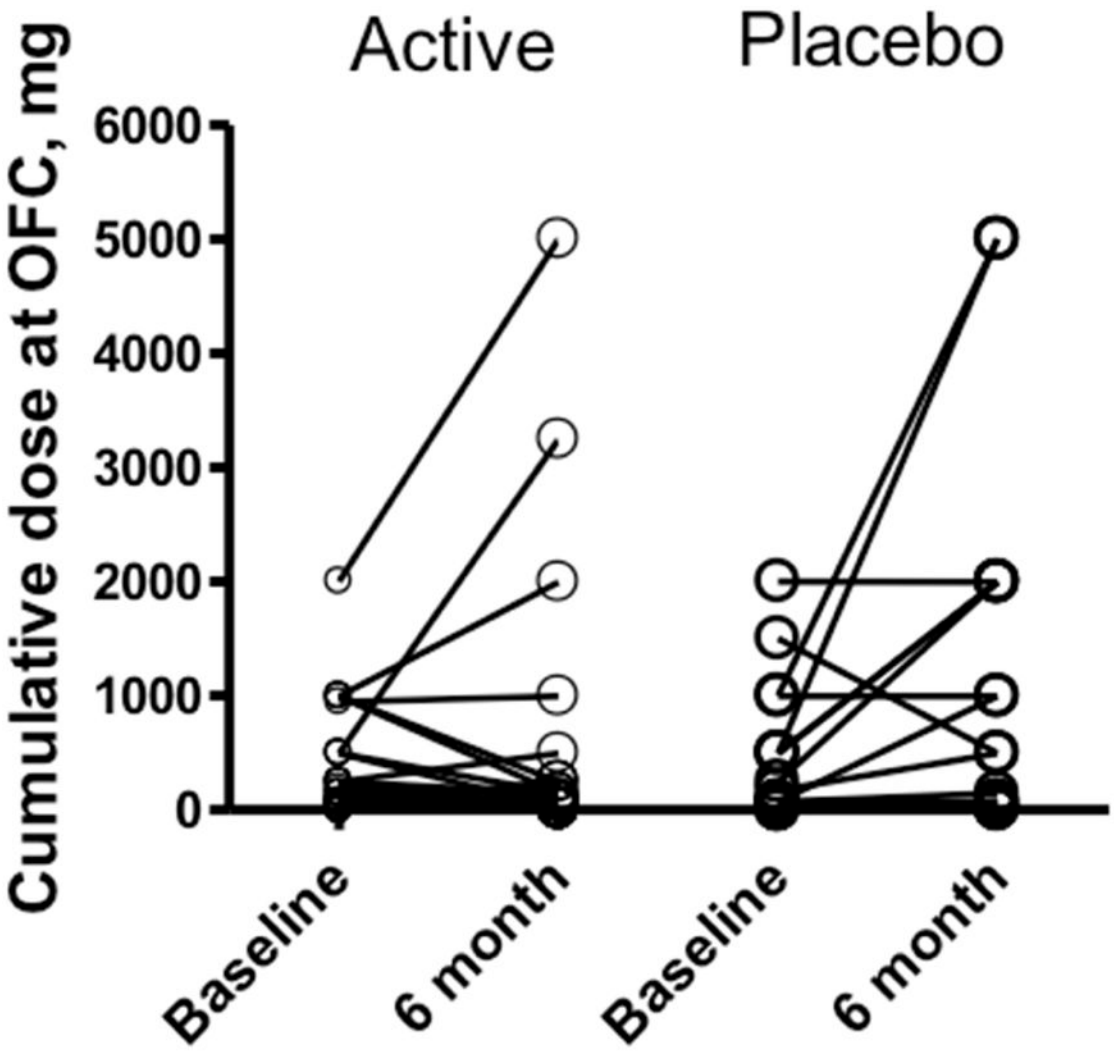

Figure 2.

Cumulative tolerated at oral food challenge at baseline and after the 6 month treatment period comparing active and placebo treated groups. The mean cumulative dose tolerated by the active group at baseline was 266.2 mg (95% CI, 150.3-382.1) and at 6 months was 369.2 mg (95% CI, 47.0-691.4). The mean cumulative dose tolerated by the placebo group at baseline was 352.5 mg (95% CI, 108.5-596.5) and at 6 months was 1022.lmg (95% CI, 326.4-1717.8).

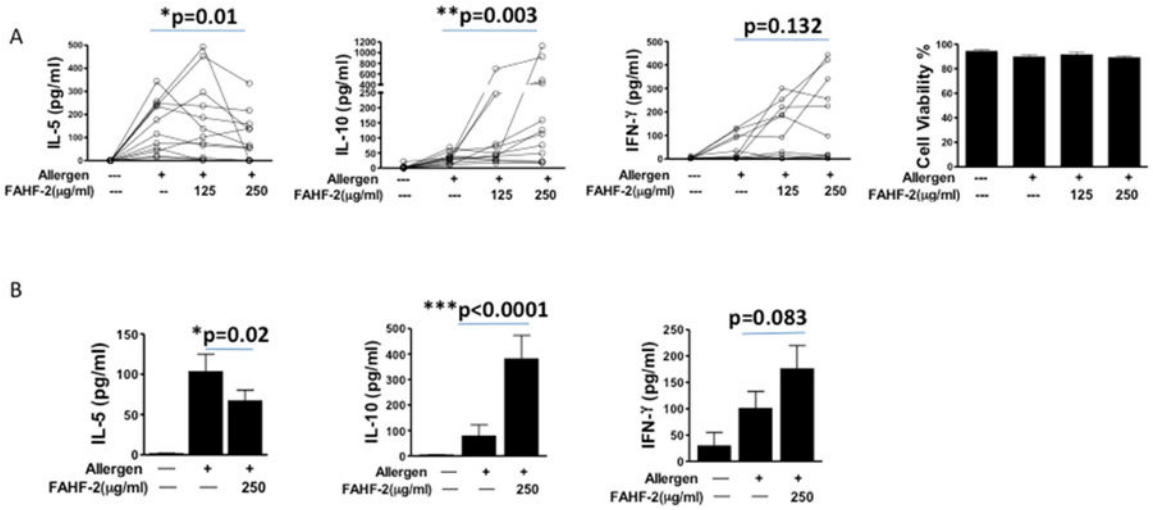

Figure 3.

FAHF-2 suppressed IL-5 and increased IL-10 *in vitro*. A. PBMCs (4×10^5) from subjects (n=12) were obtained at the baseline visit and cultured in AIM-V media alone, with relevant allergen (200μ/ml), allergen+FAHF-2 (125 or 250μg/ml). After a 3 day culture, culture supernatants were harvested and IL-5, IFN-γ and IL-10 levels were measured by ELISA. Cell viability was determined by trypan blue dye exclusion. B: IL-5 IL-10 and IFN-γ production from PBMCs obtained at baseline with or without FAHF-2 *in vitro* culture (n=53). Cultures were conducted as described in Fig 3A. Data are shown as mean ± SEM and analyzed using a left censored log-normal repeated measures tobit model. *$P < .05$, Allergen versus Allergen+FAHF-2.

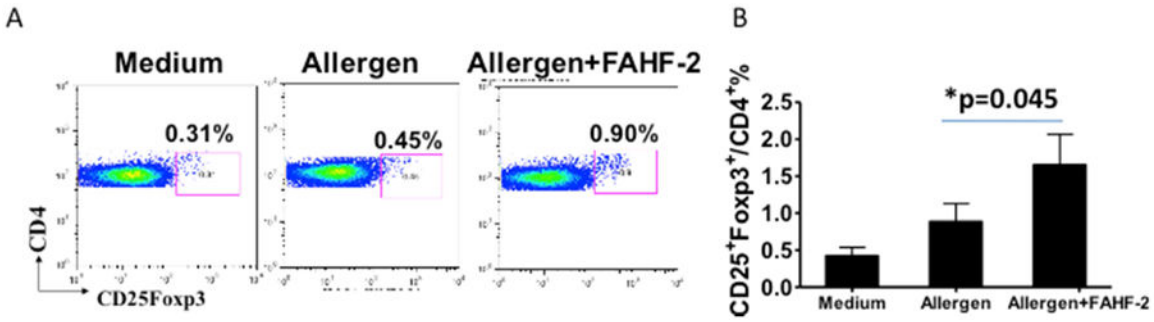

Figure 4.

FAHF-2 increased number of T regulatory cells *in vitro*. PBMCs from subjects obtained at baseline were cultured as described in Figure 2. After a 3 day culture, numbers of CD4+CD25+ Foxp3+ Tregs were determined by flow cytometry. Data were analyzed by using FlowJo software. **A,** Dot plots are representative of an individual subject. **B,** Bar graph showing data for 10 subjects. Data are shown as mean + SEM and analyzed by a mixed model with a random intercept on natural log transformed Treg percentages in 3B. *$P < .05$, Allergen versus Allergen+FAHF-2.

Table 1

Study subject characteristics

Characteristics	Active (n=46)	Placebo (n=22)	p-value
Age (median, range)	17 years (13-44 yrs)	15.5 years (12-41 yrs)	0.81
Gender – Male	27 (58.7%)	15 (68.2%)	0.60
Race			
Caucasian	42 (91.3%)	17 (77.3%)	0.27
African American	1 (2.2%)	2 (9.1%)	0.24
Asian	3 (6.5%)	3 (13.6%)	0.38
Peanut allergy	34 (73.9%)	16 (72.7%)	1
Multiple Food Allergies	39 (84.8%)	21 (95.5%)	0.26
History of anaphylaxis	19 (41.3%)	7 (31.8%)	0.60
Asthma	37 (80.4%)	13 (59.1%)	0.08
Allergic rhinitis	32 (69.6%)	16 (72.7%)	1
Atopic dermatitis	24 (52.2%)	11 (50%)	1
sIgE (median, range) (kU/L)	30.8 (0.77 →100)	20.05 (0.59 →100)	0.34
skin prick test (median, range) (mm wheal)	10.75 (3-28)	8.88 (0-26)	0.13
Baseline DBPCFC (mg protein):			
Eliciting dose	6 (1-496)	1 (1-496)	0.79
Cumulative dose	113.5 (1-2000)	71 (1-2000)	0.97
Epinephrine administered	20 (43.4%)	9 (40.9%)	0.84
Final DBPCFC (mg protein):	N=37 (9 missing)	N=21 (1 missing)	
Eliciting dose	6 (1-5000)	21 (1-3256)	0.09
Cumulative dose	21 (1-5000)	146 (1-5000)	0.07
Epinephrine administered	13 (35.1%)	4 (19.0%)	0.55 (ITT)

Table 2

Adherence to study medication for the 59 subjects who completed the 6 month trial

a. An increasing number of subjects had poor adherence over time

Assessments made at each study visit	# subjects with adherence <80%	Percentage with adherence <80%
Month 2	8	13%
Month 4	11	18.6%
Month 6	19	32%

b. Adherence to study medication was similar between active and placebo groups			
Non-adherence	**Active (n=38)**	**Placebo (n=21)**	**P-value**
Non-adherence noted at 1 study visit	9 (24%)	7 (33%)	0.54
Non-adherence noted at 2 study visits	5 (13%)	3 (14%)	1
Non-adherence noted at all study visits	0	2 (10%)	0.12
Non-adherence noted for either part or all of the 6 month study	14 (37%)	12 (57%)	0.17

Table 3

Adverse events

Adverse events	Active	Placebo	p-value
Number of AEs reported per subject – median (range)	4 (0-34)	5 (0-19)	0.88
Type of AE:			
GI	61 (23%)	28 (23%)	1
Cutaneous	17 (6.4%)	6 (4.9%)	0.65
Respiratory	56 (21.1%)	22 (18%)	0.59
Ocular	4 (1.5%)	1 (0.8%)	1
Sinusitis	4 (1.5%)	2 (1.6%)	1
Pharyngitis	15 (5.7%)	13 (10.7%)	0.09
Headache	29 (11%)	8 (6.6%)	0.20
Lab abnormality	11 (4.2%)	3 (2.5%)	0.56
Food allergic reaction	28 (10.6%)	17 (13.9%)	0.39
SCIT reaction for AR	0	3 (2.5%)*	0.03
Fever, no other symptoms	2 (0.8%)	1 (0.8%)	1
Other**	38 (14.3%)	18 (14.8%)	1
Severity:			
Mild	250	113	
Moderate	15	9	0.50
Severe	0	0	
Relatedness:			
Definitely	1	0	
Probably	7	0	
Possibly	45	18	0.66
Unrelated	212	104	0.26

* Reported for a single subject

** Other symptoms included: tooth infection, yeast infection, neck pain, orthopedic injury, ingrown toenail, ingrown hair, epistaxis, general malaise, insect bite, cold sore, otitis externa, black eye, hot flashes, sun sensitivity, difficulty sleeping, UTI, salivary gland infection, fatigue, concussion, pain from braces, thrush

Table 4

Summary of changes in immunologic parameters for active compared to placebo groups.

	Group	Baseline Visit Mean (SD)	Last Visit Mean (SD)	Baseline/Site Adjusted Difference	P-value for Diff=0	Diff between Differences [95% CI] p-value
Food Specific IgE	**A (n=27)**	23.80 (24.39)	30.55 (30.59)	6.63	0.0662	3.67 [-7.54, 14.89] P=0.51
	P (n=15)	20.16 (25.46)	24.45 (29.50)	2.96	0.5418	
Food Specific IgG4	**A (n=25)**	1.13 (1.44)	1.20 (1.67)	0.06	0.7559	0.28 [-0.34, 0.91] P=0.37
	P (n=14)	1.32 (2.06)	1.08 (1.80)	-0.22	0.3731	
Food Specific IgE/IgG$_4$ Ratio	**A (n=16)**	117.91 (227.01)	84.29 (105.67)	-27.25	0.4944	-52.66 [-183.67, 78.35] P=0.41
	P (n=10)	93.19 (223.95)	128.80 (242.98)	25.41	0.6137	
IL5 -Allergen	**A (n=30)**	342.46 (598.34)	565.01 (554.51)	211.64	0.0476	318.72 [3.75, 633.69] P=0.05
	P (n=18)	211.40 (438.91)	238.42 (285.18)	-107.08	0.4480	
IL10 – Allergen	**A (n=32)**	68.53 (129.28)	92.11 (192.53)	4.64	0.8723	34.15 [-54.43, 122.74] P=0.44
	P (n=18)	200.91 (521.89)	115.92 (172.04)	-29.51	0.4467	
IFN – γ Allergen	**A (n=32)**	156.13 (263.92)	196.92 (323.94)	78.65	0.1571	-110.00 [-281.31, 61.30] P=0.20
	P (n=17)	151.23 (230.93)	228.35 (371.57)	188.66	0.0161	