Background: Food Allergy Herbal Formula-2 (FAHF-2) is a 9-herb formula based on traditional Chinese medicine that blocks peanut-induced anaphylaxis in a murine model. In phase I studies FAHF-2 was found to be safe and well tolerated. Objective: We sought 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 aged 12 to 45 years with allergies to peanut, tree nut, sesame, fish, and/or shellfish, which were confirmed by baseline double-blind, placebo-controlled oral food challenges (DBPCFCs), received FAHF-2 (n = 46) or placebo (n = 22). After 6 months of therapy, subjects underwent DBPCFCs. For those who demonstrated increases in the eliciting dose, a repeat DBPCFC was performed 3 months after stopping therapy. Results: Treatment was well tolerated, with no serious adverse events. By using intent-to-treat analysis, the placebo group had a higher eliciting dose and cumulative dose (P = .05) at the end-of-treatment DBPCFC. There was no difference in the requirement for epinephrine to treat reactions (P = .55). There were no significant differences in allergen-specific IgE and IgG4 levels, cytokine production by PBMCs, or basophil activation between the active and placebo groups. In vitro immunologic studies performed on subjects’ baseline PBMCs incubated with FAHF-2 and food allergen produced significantly less IL-5, greater IL-10 levels, and increased numbers of regulatory T cells 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 subjects with food allergy and shows favorable in vitro immunomodulatory effects; however, efficacy for improving tolerance to food allergens is not demonstrated at the dose and duration used. (J Allergy Clin Immunol 2015;136:962-70.)

Key words: Food allergy, Food Allergy Herbal Formula-2, Chinese herbal therapy, peanut allergy

Food allergy affects as many as 8% of young children and 5% of adults. Peanut allergy is the leading cause of food-induced anaphylaxis in the United States. The standard of care for food allergy management entails strict avoidance and immediate treatment with epinephrine. Benefits from a medical management approach include fewer emergency department visits and hospitalizations, less use of rescue medications, and fewer allergic reactions with escalating doses of the allergen. A safer treatment is needed to help patients achieve greater control over their allergy and to reduce the burden of food allergy. The purpose of this study was to evaluate the safety and effectiveness of FaHF-2, a Chinese herbal medicine, in food allergy management. FaHF-2 was studied in adults and children ages 12 to 45 years treated for peanut, tree nut, sesame, fish, or shellfish sensitivities in double-blind, placebo-controlled oral food challenges (DBPCFCs).

From the Jaffe Food Allergy Institute and the Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York; the Department of Pediatrics, Division of Allergy and Immunology, University of Arkansas for Medical Sciences and Arkansas Children’s Hospital, Little Rock; and Ann & Robert H. Lurie Children’s Hospital of Chicago.

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Clinical Trial Registration: Therapeutic Effect of Chinese Herbal Medicine on Food Allergy (FAHF-2); ClinicalTrials.gov Identifier: NCT00602160.

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Abbreviations used

AE: Adverse event
DBPCFC: Double-blind, placebo-controlled oral food challenge
FAHF-2: Food Allergy Herbal Formula-2
FDA: US Food and Drug Administration
FoxP3: Forkhead box protein 3
IgE: Allergen-specific IgE
IgG4: Allergen-specific IgG4
SPT: Skin prick test
Treg: Regulatory T

access to rescue medications, and currently, there is no effective therapy or cure.

Traditional Chinese medicine has been used in China to treat various diseases for thousands of years, particularly in the form of herbal formulas. Recently, traditional Chinese medicine has been attracting interest in Western countries as a source of alternative or complementary therapy for a variety of diseases, including allergies and asthma. FAHF-2 is the first botanical investigational new drug 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. Murine model studies, as well as phase 1 acute and extended trials of FAHF-2, in subjects 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.

Therefore the aim of this study was to examine the safety and efficacy of FAHF-2 for the treatment of food allergy in a multicenter, randomized, double-blind, placebo-controlled clinical trial. On the basis of 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 cytokine production. Thus a secondary aim was to examine the immunomodulatory effects of FAHF-2 in human subjects.

METHODS

Study participants

Subjects aged 12 to 45 years with a convincing history of allergy to peanut, tree nut (almond, cashew, hazelnut, pecan, pistachio, and walnut), sesame, fish (cod, tuna, salmon, and catfish), or shellfish (crab, lobster, and shrimp), as documented by a positive skin test response (mean wheal diameter ≥5 mm larger than that elicited by the mean of saline control) and/or food allergen–specific IgE level (IgE ≥0.7 kU/L) and positive double-blind, placebo-controlled oral food challenge (DBPCFC) result (total, 2 g of protein) were eligible for the study. Only 1 food allergen was chosen to be studied during the trial for each participant. Female subjects 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, and 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 of less than 80% of predicted value; drug or alcohol abuse; pregnancy or lactation; use of oralizumab; 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 US sites (Ichan School of Medicine at Mount Sinai, New York, NY; Arkansas Children’s Hospital, Little Rock, Arkansas; and Ann and Robert H. Lurie Children’s Hospital of Chicago, Chicago, Illinois).

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

Study design

This was a randomized, double-blind, placebo-controlled trial. Subjects were randomized by using a centralized computer-generated algorithm to receive FAHF-2 or placebo (2:1), 10 tablets 3 times a day, for 6 months. The primary end point was the percentage of subjects who could consume, without dose-limiting symptoms, 2 g of protein or a greater than a 4-fold increase in the allergen dose to induce a positive DBPCFC result after therapy compared with baseline. Secondary outcomes assessed the rate of adverse events (AEs), as well as immunologic parameters.

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

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

After therapy, a DBPCFC with 5 g of protein 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 end point, returned for a DBPCFC after 3 months off therapy to assess for sustained effect.

Study medication

FAHF-2 tablets (0.5 g per 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.

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

Study procedures

SPTs. End point titrated 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, and walnut), sesame, fish (cod, tuna, salmon, and catfish), or shellfish (crab, lobster, and shrimp) (Greer Laboratories, Lenoir, NC) were used. Negative (phenol-saline solution) and positive (1 mg/mL histamine base) controls were also included. SPTs were performed by pricking with a GREER Pick (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 larger than that elicited by the negative control was considered a positive response.

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

A DBPCFC result was considered positive when a subject had 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.

Immunologic studies

**Allergen-specific IgE and IgG4 measurements.** At each study visit, sIgE levels to the study food allergen were measured by using ImmunoCAP (Thermo Fisher Scientific, Waltham, Mass). At baseline and after therapy, allergen-specific IgG4 (sIgG4) levels were measured by using ImmunoCAP.

**Cytokine profiles and basophil activation.** At baseline and after therapy, serum cytokine profiles and basophil activation (*in vitro*) studies were determined in both active and placebo subjects (see the Methods section in this article’s Online Repository).14,18

*In vitro* studies were performed to assess the response to direct exposure to FAHF-2 and predict clinical outcomes. PBMCs obtained from subjects at baseline (before treatment) were incubated with FAHF-2 plus food allergen *in vitro*, and cytokine profiles and regulatory T (Treg) cell numbers were determined to correlate cellular responses to FAHF-2 with clinical outcomes (see the Methods section in this article’s Online Repository).14,18

Safety monitoring

Subjects were monitored for potential AEs based on criteria approved by the FDA that were adapted from the World Health Organization Recommendations for Grading of Acute and Subacute Toxicity.16

Statistical analysis

A sample size of 68 subjects (allowing for a 20% dropout rate) would yield 36 active and 18 placebo evaluable subjects, providing a power of 83% to detect a difference between an estimated 60% success rate in meeting the primary end point parameters set forth in the protocol, significantly more placebo-treated subjects met the primary end point of having improvements in consumed allergen dose at the posttherapy DBPCFC compared with those on treatment (45.5% success in the placebo group vs 17.4% success in the active group, \(P = .01\)). Using intent-to-treat analysis, the placebo group had a trend for higher eliciting dose and cumulative dose at the posttherapy DBPCFC (\(P = .07\), Fig 2 and Table I). There was no difference in the requirement for epinephrine to treat reactions (\(P = .55\), Table I). Adjusting for adherence also did not alter these results.

RESULTS

Subjects’ characteristics

Sixty-eight subjects were randomized; 1 withdrew within the first 4 weeks and was replaced per protocol (Fig 1). The median age of the subjects was 16 years (range, 12-44 years), and 61.7% were male (Table I). 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 I). Furthermore, the eliciting dose did not vary by food.

Clinical outcomes

Fifty-nine (86.8%) subjects completed 6 months of treatment. One did not return for the posttherapy DBPCFC, leaving 58 evaluable subjects. There was no significant difference between the active and placebo groups in terms of completing the study (37 active and 21 placebo subjects, \(P = .09\)). Based on the primary end point parameters set forth in the protocol, significantly more placebo-treated subjects met the primary end point of having improvements in consumed allergen dose at the posttherapy DBPCFC compared with those on treatment (45.5% success in the placebo group vs 17.4% success in the active group, \(P = .01\)). Using intent-to-treat analysis, the placebo group had a trend for higher eliciting dose and cumulative dose at the posttherapy DBPCFC (\(P = .07\), Fig 2 and Table I). There was no difference in the requirement for epinephrine to treat reactions (\(P = .55\), Table I). Adjusting for adherence also did not alter these results.

Subset analyses of white race (87% of subjects) and peanut allergy (74%) showed no difference between treatment groups for the primary end point of improvement in consumed allergen dose at the posttherapy DBPCFC (16.7% success in the active group vs 33.3% in the placebo group, \(P = .17\); 14.7% success in the active group vs 37.5% in the placebo group, \(P = .14\)). No differences in eliciting or cumulative doses at baseline and posttherapy DBPCFCs were observed in these subgroups.

For those meeting the primary end point, a repeat DBPCFC with 5 g of protein was performed 3 months off treatment. Eight subjects from the active group and 10 subjects from the placebo group met this criterion. Two subjects from the placebo group declined to participate in this DBPCFC. There was no significant difference between groups for persistence of effect (5/8 in the active group vs 3/10 in the placebo group; intent-to-treat analysis, \(P = .34\)).

Additional post hoc analyses were performed by using more stringent criteria as used in the National Institutes of Health–funded Consortium of Food Allergy Research study protocols, including the following: (1) must tolerate at least 500 mg of food protein if the subject tolerated 0 to 25 mg at the baseline food challenge; (2) must tolerate at least a 10-fold increase in food protein if the subject tolerated 75 to 250 mg at the baseline food challenge; and (3) must tolerate 5 g or more of food protein if the subject tolerated more than 500 mg at the
baseline food challenge. By using these criteria, there was no difference between the active and placebo groups in achieving improved tolerance (2% success in the active group vs 13.6% success in the placebo group, \( P = .08 \)). In addition, there was no difference in persistence of effect.

**Adherence to therapy**

Adherence was assessed based on the number of tablets taken (calculated based on the 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% or greater. Nonadherence increased over the course of the study; 44% of subjects had poor adherence for at least one third of the study period (Tables II and III). There was no difference in adherence between the active and placebo groups (\( P = .17 \)).

**Clinical AEs**

A total of 387 AEs were reported; none were severe. There was no difference in the number of AEs reported per subject between the active and placebo groups (Table IV). 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; \( P = .80 \)).

Nine subjects withdrew from the study (Fig 1): 4 cited difficulties with compliance, 4 had persistent abdominal complaints, and 1 had a new rash for which the subject wanted to pursue Chinese herbal treatment prescribed by the subject’s acupuncturist. All subjects who withdrew because of persistent abdominal complaints were receiving active treatment. One of these subjects was an early dropout (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 given a diagnosis of nonceliac gluten sensitivity that responded well to a gluten-free diet; the other was given a diagnosis of 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 because of scheduling conflicts.

Immunologic 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 electrocardiographic findings did not change after treatment.

Although a significant decrease in basophil activation at 200 ng/mL in the active group from baseline to the end of the study (P = .004) was observed, there was no significant difference in the change for this parameter when comparing the active and placebo groups (P = .1). There were no significant changes in sIgE, sIgG4, sIgE/sIgG4 ratio, and IL-10 and IFN-γ levels between the treatment groups (Table V). A significant increase in IL-5 levels 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 = .9859). Adjusting for adherence did not change these results.

No difference in baseline median SPT response was detected between groups (Table I). End point titration SPTs before and after treatment comparisons found a greater median change for the area under the SPT end point titration curve for the placebo group compared with the active group (P = .03).

In vitro immunomodulatory effects of FAHF-2 on PBMCs obtained at baseline

In vitro studies were performed with PBMCs obtained from subjects at baseline to assess the response to direct exposure to FAHF-2 and predict clinical outcome. In the initial experiments

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**TABLE I.** Study subjects’ characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Active group (n = 46)</th>
<th>Placebo group (n = 22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), median (range)</td>
<td>17 (13-44)</td>
<td>15.5 (12-41)</td>
<td>.81</td>
</tr>
<tr>
<td>Male sex</td>
<td>27 (58.7%)</td>
<td>15 (68.2%)</td>
<td>.60</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>42 (91.3%)</td>
<td>17 (77.3%)</td>
<td>.27</td>
</tr>
<tr>
<td>African American</td>
<td>1 (2.2%)</td>
<td>2 (9.1%)</td>
<td>.24</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (6.5%)</td>
<td>3 (13.6%)</td>
<td>.38</td>
</tr>
<tr>
<td>Peanut allergy</td>
<td>34 (73.9%)</td>
<td>16 (72.7%)</td>
<td>1</td>
</tr>
<tr>
<td>Multiple food allergies</td>
<td>39 (84.8%)</td>
<td>21 (95.5%)</td>
<td>.26</td>
</tr>
<tr>
<td>History of anaphylaxis</td>
<td>19 (41.3%)</td>
<td>7 (31.8%)</td>
<td>.60</td>
</tr>
<tr>
<td>Asthma</td>
<td>37 (80.4%)</td>
<td>13 (59.1%)</td>
<td>.08</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>32 (69.6%)</td>
<td>16 (72.7%)</td>
<td>1</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>24 (52.2%)</td>
<td>11 (50%)</td>
<td>1</td>
</tr>
<tr>
<td>sIgE (kU/L), median (range)</td>
<td>30.8 (0.77-&gt;100)</td>
<td>20.05 (0.59-&gt;100)</td>
<td>.34</td>
</tr>
<tr>
<td>SPT (mm wheal), median, (range)</td>
<td>10.75 (3-28)</td>
<td>8.88 (0-26)</td>
<td>.13</td>
</tr>
<tr>
<td>Baseline DBPCFC (mg protein):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eliciting dose</td>
<td>6 (1-496)</td>
<td>1 (1-496)</td>
<td>.79</td>
</tr>
<tr>
<td>Cumulative dose</td>
<td>113.5 (1-2000)</td>
<td>71 (1-2000)</td>
<td>.97</td>
</tr>
<tr>
<td>Epinephrine administered</td>
<td>20 (43.4%)</td>
<td>9 (40.9%)</td>
<td>.84</td>
</tr>
<tr>
<td>Final DBPCFC (mg protein): n = 37 (9 missing) n = 21 (1 missing)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eliciting dose</td>
<td>6 (1-5000)</td>
<td>21 (1-3256)</td>
<td>.09</td>
</tr>
<tr>
<td>Cumulative dose</td>
<td>21 (1-5000)</td>
<td>146 (1-5000)</td>
<td>.07</td>
</tr>
<tr>
<td>Epinephrine administered</td>
<td>13 (35.1%)</td>
<td>4 (19.0%)</td>
<td>.55 (ITT)</td>
</tr>
</tbody>
</table>

**TABLE II.** Adherence to study medication for the 59 subjects who completed the 6-month trial: an increasing number of subjects had poor adherence over time

<table>
<thead>
<tr>
<th>Assessments made at each study visit</th>
<th>No. of subjects with adherence &lt;80%</th>
<th>Percentage with adherence &lt;80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month 2</td>
<td>8</td>
<td>13%</td>
</tr>
<tr>
<td>Month 4</td>
<td>11</td>
<td>18.6%</td>
</tr>
<tr>
<td>Month 6</td>
<td>19</td>
<td>32%</td>
</tr>
</tbody>
</table>

**TABLE III.** Adherence to study medication for the 59 subjects who completed the 6-month trial: adherence to study medication was similar between the active and placebo groups

<table>
<thead>
<tr>
<th>Nonadherence</th>
<th>Active group (n = 38)</th>
<th>Placebo group (n = 21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonadherence noted at 1 study visit</td>
<td>9 (24%)</td>
<td>7 (33%)</td>
<td>.54</td>
</tr>
<tr>
<td>Nonadherence noted at 2 study visits</td>
<td>5 (13%)</td>
<td>3 (14%)</td>
<td>1</td>
</tr>
<tr>
<td>Nonadherence noted at all study visits</td>
<td>0</td>
<td>2 (10%)</td>
<td>.12</td>
</tr>
<tr>
<td>Nonadherence noted for either part or all of the 6-mo study</td>
<td>14 (37%)</td>
<td>12 (57%)</td>
<td>.17</td>
</tr>
</tbody>
</table>

*ITT, Intent-to-treat analysis.*
we tested 2 doses. As shown in [Fig 3, A], cultures with allergen plus 250 μg/mL FAHF-2 showed significantly lower levels of IL-5 and higher levels of IL-10 compared with cultures with allergen alone (n = 12). There was no difference in cytokine levels between PBMCs cultured with allergen alone and allergen plus 125 μg/mL FAHF-2. We then determined the effects of 250 μg/mL FAHF-2 and found a reduction in IL-5 levels and an increase in IL-10 levels (allergen plus FAHF-2 vs allergen alone, P < .05, n = 53; [Fig 3, B]). Significantly increased number of CD4+CD25+forkhead box protein 3 (FoxP3)+ Treg cells was exhibited under FAHF-2-treated conditions (FAHF-2 plus allergen vs allergen alone, P < .05, n = 10, [Fig 4]).

**DISCUSSION**

FAHF-2 is a 9-herb formula that is highly safe and effective in murine models of peanut and multiple food allergies. Based on the favorable results of acute and extended phase I studies, we performed a multicenter, randomized, double-blind, placebo-controlled phase II clinical trial to assess safety and efficacy in subjects with food allergy.

The results of this study did not demonstrate the efficacy of FAHF-2 by using a dose of 10 tablets 3 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 AEIs, 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 because of gastrointestinal complaints, suggesting that the number of tablets and/or herbal medications might adversely affect certain subjects. 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 might have affected our ability to detect efficacy. First, the dose used was based on an extrapolation from the effective murine dose by using body surface area while also considering the tablet burden that can negatively affect adherence. In this trial 80% of the full murine dose was chosen, which was 10 tablets 3 times a day. This high tablet load posed a significant burden on subjects, contributing to dropout, as well as low adherence. Nearly half of the subjects had less than 80% medication adherence for at least 2 months of the 6-month study, and a third were nonadherent during 4 to 6 months just before the posttherapy DBPCFC. Thus suboptimal dosing might contribute to the lack of efficacy seen in this clinical trial.

Second, the treatment duration was suboptimal. Two to 3 years of therapy would be required in human subjects to achieve a comparable duration to the 7-week treatment in the mouse. Data from oral immunotherapy (OIT) studies also indicate that longer treatment durations are likely to be more effective for well-established food allergy.

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

Consistent with previous findings, PBMCs cultured with FAHF-2 switched from antigen-induced Th2 to Th1/Treg cell–predominant responses. A higher number of CD4+CD25+FoxP3+ Treg cells were present in FAHF-2 plus antigen cultures than in cultures with allergen alone. The discrepancy between in vitro and ex vivo results might 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 might influence the ability to modulate the immune system toward tolerance. OIT and sublingual immunotherapy studies suggest that an older age at the start of treatment might result in more difficulty achieving desensitization because success rates tended to be higher in studies that included primarily younger children. Furthermore, in 2 studies that included children with a history of anaphylaxis, lower success rates for desensitization were observed in comparison with studies that excluded those with a history of anaphylaxis. Subjects in our study were older, with a median age of 16 years, and more than a third had a history of anaphylaxis; thus our study population might need optimized doses and prolonged treatment to affect their established allergy.

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**TABLE IV. AEs**

<table>
<thead>
<tr>
<th>AEs</th>
<th>Active group</th>
<th>Placebo group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of AEs reported per subject, median (range)</td>
<td>4 (0-34)</td>
<td>5 (0-19)</td>
<td>.88</td>
</tr>
<tr>
<td>Type of AE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>61 (23%)</td>
<td>28 (23%)</td>
<td>1</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>17 (6.4%)</td>
<td>6 (4.9%)</td>
<td>.65</td>
</tr>
<tr>
<td>Respiratory</td>
<td>56 (21.1%)</td>
<td>22 (18%)</td>
<td>.59</td>
</tr>
<tr>
<td>Ocular</td>
<td>4 (1.5%)</td>
<td>1 (0.8%)</td>
<td>1</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>4 (1.5%)</td>
<td>2 (1.6%)</td>
<td>1</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>15 (5.7%)</td>
<td>13 (10.7%)</td>
<td>.09</td>
</tr>
<tr>
<td>Headache</td>
<td>29 (11%)</td>
<td>8 (6.6%)</td>
<td>.20</td>
</tr>
<tr>
<td>Laboratory abnormality</td>
<td>11 (4.2%)</td>
<td>3 (2.5%)</td>
<td>.56</td>
</tr>
<tr>
<td>Food-induced allergic reaction</td>
<td>28 (10.6%)</td>
<td>17 (13.9%)</td>
<td>.39</td>
</tr>
<tr>
<td>SCIT reaction for AR</td>
<td>0</td>
<td>3 (2.5%)*</td>
<td>.03</td>
</tr>
<tr>
<td>Fever, no other symptoms</td>
<td>2 (0.8%)</td>
<td>1 (0.8%)</td>
<td>1</td>
</tr>
<tr>
<td>Other†</td>
<td>38 (14.3%)</td>
<td>18 (14.8%)</td>
<td>1</td>
</tr>
</tbody>
</table>

**Severity**

- Mild
  - Active group: 250
  - Placebo group: 113
  - P value: .50
- Moderate
  - Active group: 15
  - Placebo group: 9
  - P value: .50
- Severe
  - Active group: 0
  - Placebo group: 0

**Relatedness**

- Definitely
  - Active group: 1
  - Placebo group: 0
  - P value: .05
- Probable
  - Active group: 7
  - Placebo group: 0
  - P value: .05
- Possibly
  - Active group: 45
  - Placebo group: 18
  - P value: .66
- Unrelated
  - Active group: 212
  - Placebo group: 104
  - P value: .26

*AR, Allergic rhinitis; SCIT, subcutaneous immunotherapy.
†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, urinary tract infection, salivary gland infection, fatigue, concussion, pain from braces, and thrush.
Although 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 end point criteria and trial design. Clinically relevant end points are necessary because small improvements in cumulative dose in a positive DBPCFC do not provide sufficient protection in case of a true accidental exposure. Using our original end point parameters, significantly more placebo-treated subjects experienced improvements in tolerance after 6 months. However, post hoc analyses using the criteria from the Consortium of Food Allergy Research group showed no difference between treatment groups. This is due to several subjects being categorized as improved based on small incremental increases in the dose consumed without symptoms during the DBPCFC by using the original end point parameters, which are likely to be clinically irrelevant. This also suggests that eliciting and cumulative doses might 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 sublingual immunotherapy study. In recent peanut OIT studies the improvement rate in placebo groups varied between 0% and 15%. Spontaneous tolerance to tree nuts and fish/shellfish are generally reported to be 9% and 1% to 2%, respectively. 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 in which the chances of receiving active treatment exceed 50% because there is a high expectation of improvement, thus leading to poor discrimination between treatment groups. This study randomized subjects 2:1 to the active and placebo groups (67% chance of

<table>
<thead>
<tr>
<th>TABLE V. Summary of changes in immunologic parameters for the active compared with placebo groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Food-specific IgE</td>
</tr>
<tr>
<td>Placebo (n = 15)</td>
</tr>
<tr>
<td>Food-specific IgG4</td>
</tr>
<tr>
<td>Placebo (n = 14)</td>
</tr>
<tr>
<td>Food-specific IgE/IgG4 ratio</td>
</tr>
<tr>
<td>Placebo (n = 10)</td>
</tr>
<tr>
<td>IL-5/allergen</td>
</tr>
<tr>
<td>Placebo (n = 18)</td>
</tr>
<tr>
<td>IL-10/allergen</td>
</tr>
<tr>
<td>Placebo (n = 18)</td>
</tr>
<tr>
<td>IFN-γ/allergen</td>
</tr>
<tr>
<td>Placebo (n = 17)</td>
</tr>
</tbody>
</table>

**Diff.** Difference.

**FIG 3.** FAHF-2 suppressed IL-5 and increased IL-10 levels in vitro. A, PBMCs (4 × 10⁵) from subjects (n = 12) were obtained at the baseline visit and cultured in AIM-V media alone, with relevant allergen (200 μg/mL) or allergen plus FAHF-2 (125 or 250 μg/mL). After a 3-day culture, culture supernatants were measured by means of ELISA. Cell viability was determined by using 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 3, A. Data are shown as means ± SEMs and analyzed by using a left-censored, log-normal, repeated-measures tobit model. *P < .05, allergen versus allergen plus FAHF-2; **P < .01; and ***P < .001.
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 because of a high likelihood of receiving placebo treatment. In retrospect, this study design might have contributed to our observation that more placebo-treated subjects experienced an increase in cumulative tolerated dose at the posttherapy DBPCFC.

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

We thank Jaime Ross, RN; Paul Faybusovich; Min-Li Hong; June Straw, RN; Suzanne Carlisle, RN; Maripaz Vazquez, RN, BSN; and Wendy Lankenau, RN, for study coordination and support. We thank the staff of the clinical research unit at each institution and the subjects and families who kindly participated.

Key messages

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

REFERENCES

METHODS

**Determination of cytokine profiles in subjects before and after treatment (ex vivo study)**

PBMCs from subjects were separated on a Ficoll gradient (GE Healthcare, Piscataway, NJ). For the *ex vivo* study, 4 × 10^5 cells per well were cultured in AIM-V medium (Invitrogen, Carlsbad, Calif) in either medium alone or with allergen (200 µg/mL) or PHA (2 µg/mL, Invitrogen) in 96-well round-bottom plates. The cells were incubated in tissue-culture conditions for 72 hours at baseline and after 6 months of treatment with FAHF-2 or placebo. Crude antigen extracts were generated in the laboratory at Mount Sinai, as previously described.\(^1\) The supernatants were analyzed for the presence of IL-5, IFN-γ, and IL-10 levels by means of ELISA (BD Biosciences, San Diego, Calif).

**Basophil activation test**

Basophil activation tests were performed with the Flow 2 CAST kit (Alpco Diagnostics, Windham, NH), as previously described.\(^2\) Crude allergen extracts were used as specific allergens for *ex vivo* stimulation.\(^1\) A stimulation buffer containing IL-3 was used as the negative control. Anti-FcεRI mAb and N-formyl-methionyl-leucyl-phenylalanine were used as positive controls, per the manufacturer’s instructions. One hundred microliters of stimulation buffer containing IL-3, heparin, and calcium was added to six 50-µL aliquots of heparinized blood. Fifty microliters of stimulation buffer, anti-FcεRI, N-formyl-methionyl-leucyl-phenylalanine, or allergen (200 ng/mL, 2 ng/mL, and 20 pg/mL) was added to each aliquot. Twenty microliters of staining reagent containing anti-CCR3–fluorescein isothiocyanate–labeled and anti-CD63–phycoerythrin–labeled mAbs was added. The tubes were then incubated at 37°C in a water bath in the dark for 15 minutes. Red blood cells were lysed, and cells were resuspended in wash buffer and acquired on an LSR II flow cytometer (BD Biosciences). Basophils and eosinophils were gated as CCR3\(^{+}\) cells and segregated on the basis of side scatter. CCR3\(^{+}\) cells with low side scatter were considered basophils, and among these, the CD63\(^{+}\) cells were termed activated basophils. Fifty thousand to 100,000 leukocytes were acquired, and 300 or more basophils were used for analysis with FlowJo software (Tree Star, Ashland, Ore).

**Determine cytokine profiles in PBMCs and Treg cells in response to *in vitro* FAHF-2 treatment**

For the *in vitro* study of the direct effect of FAHF-2 on cytokine profiles, PBMCs were obtained at baseline (sufficient samples from 53 subjects were available after the *ex vivo* experiments [male/female, 3:2; age, 18 ± 7 years]). PBMCs were cultured with AIM-V media alone, allergen (200 µg/mL), allergen plus FAHF-2 (125 or 250 µg/mL), or PHA (2 µg/mL) for 72 hours, as previously described.\(^1\),\(^2\) Supernatants were harvested, and cytokine levels were measured by means of ELISA. Aliquots of cells (4 × 10^6 per condition) were stained with anti-CD4–allophycocyanin and anti-CD25–phycoerythin, followed by fixation, permeabilization, and incubation with the anti-FoxP3–fluorescein isothiocyanate antibody. Cells were acquired on an LSR II flow cytometer and analyzed with FlowJo software. CD4\(^{+}\)CD25\(^{+}\)FoxP3\(^{+}\) cells were identified as Treg cells.

REFERENCES
