

The Chinese herbal medicine formula FAHF-2 completely blocks anaphylactic reactions in a murine model of peanut allergy

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Background: Peanut allergy is potentially life threatening. There is no curative therapy for this disorder. We previously found that an herbal formula, food allergy herbal formula (FAHF)–1, blocked peanut-induced anaphylaxis in a murine model when challenged immediately posttherapy.

Objective: To test whether FAHF-2, an improved herbal formula, from which 2 herbs, *Zhi Fu Zi* (Radix Lateralis Aconiti Carmichaeli Praeparata) and *Xi Xin* (Herba Asari), were eliminated, is equally effective to FAHF-1, and if so, whether protection persists after therapy is discontinued. **Methods:** Mice allergic to peanut treated with FAHF-2 for 7 weeks were challenged 1, 3, or 5 weeks posttherapy. Anaphylactic scores, core body temperatures, vascular leakage, and plasma histamine levels after peanut challenge were determined. Serum peanut-specific antibody levels and splenocyte cytokine profiles were also measured.

Results: After challenges, all sham-treated mice developed severe anaphylactic signs, significant decrease in rectal temperatures, significantly increased plasma histamine levels, and marked vascular leakage. In contrast, no sign of anaphylactic reactions, decrease in rectal temperatures, or elevation of plasma histamine levels was observed in FAHF-2–treated mice in 5 separate experiments. IgE levels were significantly reduced by FAHF-2 treatment and remained significantly lower as long as 5 weeks posttherapy. Splenocytes from FAHF-2–treated mice showed significantly reduced IL-4, IL-5, and IL-13, and enhanced IFN- γ production to recall peanut stimulation *in vitro*.

Conclusion: FAHF-2 treatment completely eliminated anaphylaxis in mice allergic to peanut challenged as long as 5 weeks posttherapy. This result was associated with downregulation of T_H2 responses. FAHF-2 may be a potentially

effective and safe therapy for peanut allergy. (J Allergy Clin Immunol 2005;115:171-8.)

Key words: Peanut anaphylaxis, Chinese herbal medicine formula, T_H2 cytokine modulation

Peanut anaphylaxis, an IgE-mediated type I hypersensitivity, accounts for 2/3 of food-induced fatal anaphylactic shock cases.¹ The prevalence of childhood peanut allergy (PNA) doubled between 1997 and 2002, and peanut and tree nut allergies currently affect more than 3 million Americans.² Currently, the only way to manage PNA is strict avoidance, but because peanut is a hidden ingredient in several processed foods, accidental ingestions are common.³ Although most food allergies are outgrown, PNA is usually lifelong.⁴ PNA has a severe negative effect on the quality of life of affected children and their families.⁵ In the past 5 years, much effort has been devoted to developing a treatment for PNA.^{6,7} A recent clinical trial showed that monthly injections of humanized recombinant anti-IgE antibodies increased the threshold for allergic responses of peanut-sensitive subjects, at least to small amounts of peanut protein.⁸ However, continuous monthly injections are necessary to maintain protection. Alternative therapies for the treatment of peanut allergy are needed. Our murine model of peanut anaphylaxis, which physiologically and immunologically mimics PNA in human beings, has been used to develop novel approaches for PNA.^{7,9-11}

Chinese herbal medicines have been suggested to be of potential value for treating allergies.^{12,13} We previously reported that an herbal formula, food allergy herbal formula (FAHF)–1, developed in our laboratory, blocked systemic anaphylactic signs and reduced mast cell degranulation and histamine release, peanut-specific serum IgE level, and T_H2 cytokine secretion in a murine model of PNA.¹⁴ FAHF-1 is nontoxic in an animal model.¹⁴ These results suggested that herbal medicines may have potential for developing novel therapies for PNA.

The complexity of traditional Chinese herbal formulas containing many constituents makes standardization of herbal products difficult. This report describes our initial attempt to simplify FAHF-1 while maintaining therapeutic efficacy. We eliminated 2 herbs—*Zhi Fu Zi* (Radix Lateralis Aconiti Carmichaeli Praeparata) and *Xi Xin* (Herba Asari)—from FAHF-1 because they are not likely to be the principal herbs on the basis of the Traditional

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US Provisional Patent Application (reference #60554775) regarding FAHF-2 has been filed.

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Abbreviations used

BUN: Blood urea nitrogen
 CBC: Complete blood cell count
 ConA: Concanavalin A
 FAHF: Food allergy herbal formula
 PNA: Peanut allergy

Chinese Medicine formulation system¹⁵ and may be toxic if incorrectly processed or overdosed.¹⁶⁻¹⁹ Furthermore, the US Food and Drug Administration has expressed concern over residual aconitine in *Zhi Fu Zhi* and adulteration of *Xi Xin* with aristolochic acid. We named this refined formula FAHF-2. In a preliminary study, we compared the efficacy of FAHF-2 with FAHF-1 on peanut anaphylaxis by using the original protocol,¹⁴ in which mice were challenged with peanut immediately after completing treatment (posttherapy), and found that FAHF-2 also completely blocked anaphylaxis after peanut challenge (data not shown). Here, we report the results of our extended study in which mice allergic to peanut were challenged 1 week, 3 weeks, or 5 weeks posttherapy in a series of 5 experiments to determine whether FAHF-2 treatment effects persisted. We found that FAHF-2-treated mice were completely protected from anaphylaxis after challenge for as long as 5 weeks posttherapy, and that reduced IgE levels remained significantly lower at 5 weeks posttherapy. This therapeutic effect was associated with immunoregulatory effects on T_H1-T_H2 responses. This study may lead to an herbal therapy for PNA.

METHODS**Mice and reagents**

Five-week-old female C3H/HeJ mice purchased from the Jackson Laboratory (Bar Harbor, Me) were maintained on peanut-free chow under specific pathogen-free conditions according to standard guidelines for the care and use of animals.²⁰ Freshly ground whole roasted peanut and crude peanut extract prepared as previously described^{9,21,22} were used as antigens. Reagents were purchased from the following sources: cholera toxin, List Biological Laboratories, Campbell, Calif; concanavalin A (ConA) and albumin-dinitrophenyl, Sigma, St Louis, Mo; antibodies for ELISAs (sheep antimouse IgE, and biotinylated donkey antisheep IgG), Binding Site, San Diego, Calif; and antidinitrophenyl IgE, IgG2a, and IgG1, Accurate Scientific, Westbury, NY. FAHF-2 formula contains 9 herbs (Table I). Dried extract of FAHF-2, produced in a good manufacturing practice-certified facility, was obtained from Beijing Shen Hua Shi Di Medical Technology, Beijing, China, and stored at room temperature. The quality of the raw herbs was controlled according to the requirements of Pharmacopoeia of People's Republic of China.²³ The same batch of product (batch #20020105) was used in all experiments in this study.

Intragastric peanut sensitization, challenge, and FAHF-2 treatment

Mice were sensitized intragastrically with ground peanut (10 mg/mouse) and cholera toxin (20 µg/mouse) weekly for 3 weeks as

previously described,¹⁴ then treated intragastrically with FAHF-2 (20 mg/mouse in 0.5 mL water) twice daily for 7 weeks (weeks 3-9). This dose was based on a conversion table of equivalent effective dose ratios from human beings to animals based on body surface area.²⁴ During treatment, mice were boosted at weeks 5 and 7 with ground peanut (50 mg/mouse) plus cholera toxin (20 µg/mouse) to maintain hypersensitivity. Water (sham)-treated and naive mice served as controls. All groups of mice were challenged with ground peanut (200 mg/mouse) at week 10 (1 week posttherapy; Table II; experiment #1). To determine whether FAHF-2 protected against anaphylaxis for a significant period of time posttherapy, mice received 6 sensitization doses at weekly intervals followed by 2 boosting doses at weeks 6 and 8, as previously described.²⁵ The treatment regimen was the same as above. Mice were challenged at week 12 or 14 (3 and 5 weeks posttherapy; Table II; experiments #2-5). Our previous study²⁵ showed that after the last boost (at week 8), week 14 was the latest time at which mice still exhibited hypersensitivity to initial challenge.

Assessment of systemic anaphylactic signs

Anaphylactic signs were evaluated 30 to 40 minutes after the second challenge dose by 2 investigators by using the scoring previously described^{9,14}: 0, no signs; 1, scratching and rubbing around the snout and head; 2, puffiness around the eyes and snout, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth and the tail; 4, no activity after prodding, or tremor and convulsions; and 5, death.

Measurement of rectal temperature

Rectal temperatures were measured 25 minutes after challenge by using a thermal probe (Harvard Apparatus, Newark, NJ).

Measurement of plasma histamine levels

Plasma histamine levels were obtained 30 minutes after the second challenge and analyzed by using an enzyme immunoassay kit (ImmunoTECH, Marseille, France) as described by the manufacturer.²⁶

Detection of vascular leakage

Immediately before the second intragastric peanut challenge dose, 2 mice from each group received 100 µL 0.5% Evan's blue dye by tail vein injection. Footpads of mice were examined for signs of vascular leakage (visible blue color) 30 to 40 minutes after dye/antigen administration as previously described.²⁶

Measurement of serum peanut-specific IgE, IgG1, and IgG2a levels

Venous blood samples were obtained from tail veins 1 day before treatment, 1 day before boosting, and 1 day before challenge. Sera were collected and stored at -80°C until analyzed. Levels of peanut-specific IgE, IgG1, and IgG2a levels were determined by ELISA as previously described.⁹

Cell culture and cytokine measurements

Pooled splenocytes from each group prepared after challenge as previously described^{25,27} were cultured in 24-well plates (4 × 10⁶/well/mL) in the presence of 200 µg/mL crude peanut extract (PN), 2.5 µg/mL Concanavalin A (ConA), or medium alone (Med). Supernatants were collected after 72 hours of culture. Cytokine levels were determined by ELISA in triplicate according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn, for IL-13; PharMingen, San Diego, Calif, for all others).

TABLE I. Components of herbal medicines in FAHF-2*

Name of TCM Materia Medica (Pin Yin)	Equivalent pharmaceutical name	Part used	Amount (% of total)
Ling Zhi (Chi)	Ganoderma Lucidum	Fruiting body	28.17
Wu Mei	Fructus Pruni Mume	Fruit	28.17
Chuan Jiao	Pericarpium Zanthoxyli Bungeani	Seed	1.41
Huang Lian (Chuan)	Rhizoma Coptidis	Root	8.46
Huang Bai	Cortex Phellodendri	Root	5.63
Gan Jiang	Rhizoma Zingiberis Officinalis	Root	8.45
Gui Zhi	Ramulus Cinnamomi Cassiae	Twig	2.81
Ren Shen (Hong)	Radix Ginseng	Root	8.45
Dang Gui (Shen)	Corpus Radix Angelicae Sinensis	Root	8.45

TCM, Traditional Chinese Medicine

*All of the herbs are of Chinese origin.

TABLE II. Clinical signs of anaphylactic reactions after oral peanut challenge

Experiment	Challenge time	Sham		FAHF-2		Naive	
		N/total	Anaphylactic score, median (range)	N/total	Anaphylactic score, median	N/total	Anaphylactic score, median
1	Week 10	9/9	3 (2-4)	0/9	0***	0/9	0
2	Week 12	8/8	3 (2-4)	0/4	0***	0/5	0
3	Week 14	8/8	3 (2-3)	0/4	0**	0/5	0
4	Week 14	8/8	3 (2-4)	0/4	0***	0/5	0
5	Week 14	5/5	3 (2-3)	0/5	0***	0/5	0
Totals		38/38	3 (2-4)	0/26	0###	0/29	0

Mice in each group were challenged intragastrically with peanut at week 10 after the initial peanut sensitization (1 week posttherapy), week 12 (3 weeks posttherapy), or week 14 (5 weeks posttherapy). Clinical signs of anaphylaxis were evaluated 30 minutes after challenge. Anaphylactic scores were determined as described in Methods.

** $P < .01$ and *** $P < .001$ vs sham in individual experiments; ### $P < .001$ vs sham in totals.

Safety testing

LD50 evaluation. The Lethal Dose 50 (LD50) protocol was designed as follows. Mice were fed 12 times the therapeutic mouse daily dose of FAHF-2 and observed for 12 hour and 24 hours and then for 7 days. If there were fewer than 50% deaths, an additional group of mice was given a higher dose, and all surviving mice were kept for 14 days. Biochemical analyses, complete blood cell count (CBC), and histological analysis were then conducted. This protocol was continued until either an LD50 was established or the absolute dose limitation was reached. The absolute dose limitation of FAHF-2 is 24 times the effective dose. Mice fed with water served as controls (sham).

Biochemical analysis and CBC testing. Biochemical analyses of blood urea nitrogen (BUN), creatinine, and alanine aminotransferase (ALT) to assess kidney and liver functions, respectively, were performed by using PROCHEM-V instrumentation (Synbiotics Co, San Diego, Calif). For CBC testing, blood samples (20 μ L) were collected and subjected to analysis by Multispecies Hematology Systems (CDC Technologies, Oxford, Conn). These assays were performed at Mount Sinai School of Medicine, Center for Laboratory Animal Sciences, where these assays are routinely performed to monitor the health of laboratory animals.

Histology. Histological analysis of major organs (kidney, liver, heart, spleen, lung, stomach, and intestine) was performed in a blind manner.

Statistical analysis

Data were analyzed by using the SigmaStat statistical software package (SPSS, Chicago, Ill). For anaphylactic scores, rectal temperature, and histamine levels, the differences between the groups were analyzed by the Kruskal-Wallis 1-way ANOVA on ranks followed by the Mann-Whitney rank-sum test because the data failed to pass the normality test. For IgE, IgG2a, and cytokine levels, the differences between the groups were analyzed by 1-way ANOVA followed by the Bonferroni t test for all pairwise comparisons because the data passed the normality test. P values $< .05$ were considered significant.

RESULTS

FAHF-2 treatment completely prevented symptoms of anaphylactic reactions after oral peanut challenge

After challenge at week 10 (1 week posttherapy), all sham-treated mice developed anaphylactic reactions (median score, 3; Table II; experiment #1). In contrast, FAHF-2-treated mice exhibited no anaphylactic signs. The same results were found after week 12 and 14 challenges (Table II; 3 and 5 weeks posttherapy; experiments #2-5).

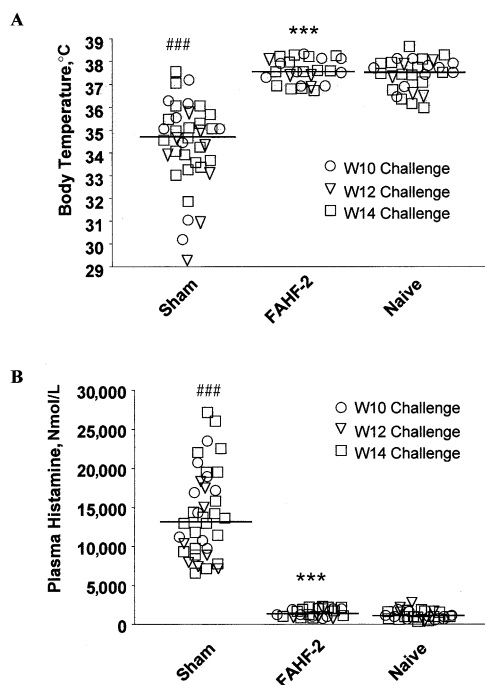


FIG 1. A, FAHF-2 prevented a drop in core body temperatures. Rectal temperatures were measured 25 minutes after intragastric peanut challenge in mice challenged at weeks 10 (○), 12 (▽), and 14 (□). Bars indicate the medians of temperature from totals of 5 sets of experiments (sham, $n = 38$; FAHF-2, $n = 26$; naive, $n = 29$). ### $P < .001$ vs naive; *** $P < .001$ vs sham. **B, FAHF-2 blocked histamine release.** Data are plasma histamine levels 30 minutes postchallenge for the same mice as in A. ### $P < .001$ vs naive; *** $P < .001$ vs sham.

In a series of 5 experiments, all 38 sham-treated mice developed anaphylaxis with a median anaphylactic score of 3, indicating a severe anaphylactic reaction. In contrast, 0 of 26 FAHF-2-treated mice and 0 of 29 naive mice developed anaphylaxis after challenge. Statistical significances in anaphylactic scores between the FAHF-2-treated and the sham-treated mice were obtained from individual experiments and pooled data of 5 experiments (Table II). These results demonstrated that FAHF-2 has potent protective properties against peanut-induced anaphylaxis and that the complete protection was consistent and relatively persistent. Over the 2.5-year period in which these experiments were performed, FAHF-2 showed no detectable loss of activity.

FAHF-2 completely blocked decreases in body temperature and histamine release after oral peanut challenge

Core body temperature drops during systemic anaphylaxis. We used rectal temperature measurements 25 minutes after challenge as an objective measurement of anaphylaxis. As shown in Fig 1, A, median rectal temperature in the sham-treated mice in a series of 5 experiments, as shown in Table II, was significantly lower than that of naive mice (34.8°C vs 37.40°C ; $P < .001$), whereas

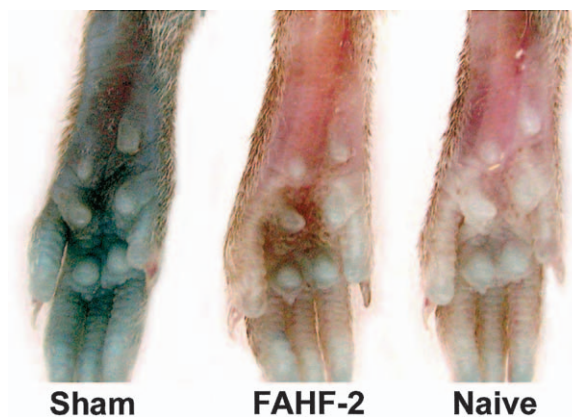


FIG 2. FAHF-2 prevented vascular leakage. Photograph illustrates mouse footpads after Evan's blue dye/peanut administration at week 14. Marked vascular leakage (blue) is seen in the sham-treated mice but not in FAHF-2-treated and naive mice. Results represent 2 to 3 mice from each group.

median temperatures in the FAHF-2-treated mice were the same as those in naive mice (37.43°C vs 37.40°C) and significantly higher than in the sham-treated mice ($P < .001$) regardless of the time of challenge posttherapy.

We previously found that anaphylactic scores in this model were associated with plasma histamine levels.⁹ Therefore, we also determined histamine levels 30 minutes after challenge. As shown in Fig 1, B, plasma histamine levels were markedly elevated in sham-treated mice compared with naive mice (sham vs naive, median, nM: 13,843 vs 1303; $P < .001$). In contrast, histamine levels in the FAHF-2-treated mice were essentially the same as in naive mice (median, nM: 1444 vs 1303) and significantly lower than in the sham-treated mice ($P < .001$). These results demonstrated that protection of FAHF-2 against peanut-induced anaphylaxis included blocking histamine release.

FAHF-2 blocked vascular leakage after oral peanut challenge

Vascular leakage mediated by histamine is a hallmark of anaphylactic shock. We also determined whether FAHF-2 blocks vascular leakage by assessing the color of footpads after Evan's blue dye/antigen administration. As illustrated in Fig 2, footpads of sham-treated mice exhibited extensive vascular leakage, shown by blue color, whereas footpads of the FAHF-2-treated group were normal. These results further support the findings that FAHF-2 eliminated clinical signs and blocked histamine release.

FAHF-2 suppressed peanut-specific IgE synthesis

Peanut hypersensitivity is IgE-mediated. We evaluated the effect of FAHF-2 on peanut-specific IgE production and found that FAHF-2-treated mice showed a 51% and 54% reduction compared with the sham-treated mice at the

time of challenge in weeks 10 and 12 challenge experiments, respectively (Table II; experiments #1 and #2). Fig 3 shows kinetic IgE levels from 3 sets of week 14 challenge experiments. Peanut-specific IgE levels were not different between groups before treatment (Fig 3, A; week 3) but were significantly lower in FAHF-2–treated mice compared with the sham-treated mice at week 8 (during treatment; $P < .001$), week 10 (1 week posttherapy; $P < .001$), and week 14 (5 weeks posttherapy; $P < .001$). These results demonstrated that the FAHF-2–induced reduction in peanut-specific IgE levels did not rebound for at least 5 weeks posttherapy. In addition, IgG2a levels in FAHF-2–treated groups were significantly higher than in the sham-treated groups at weeks 10 and 14 ($P < .001$; Fig 3, B). IgG1 levels were slightly reduced in the FAHF-2–treated group, but the difference did not reach statistical significance at any time points during treatment or posttherapy (data not shown).

FAHF-2 suppressed T_H2 responses to recall peanut stimulation

Increasing evidence suggests that PNA is T_H2 -mediated.²⁸ To begin to determine the mechanisms of FAHF-2 effects on PNA, we investigated whether FAHF-2 treatment modulates T_H2 responses by comparing splenocyte cytokine profiles in response to recall peanut stimulation *in vitro*. We found that splenocytes from FAHF-2–treated mice produced significantly lower levels of T_H2 cytokines at the times of week 10 and week 12 challenges, but the increase in IFN- γ levels did not reach statistical significance (data not shown). Fig 4 shows IL-4, IL-5, IL-13 (T_H2 cytokines), IFN- γ (T_H1 cytokine), IL-10 (a T-regulatory cytokine), and TGF- β (a T_H3 cytokine) at time of week 14 challenges. Splenocytes from FAHF-2–treated mice produced significantly less IL-4, IL-5, and IL-13, the 3 major T_H2 cytokines, than splenocytes from sham-treated mice ($P < .01$, $P < .001$, and $P < .01$, respectively). Interestingly, splenocytes from FAHF-2–treated mice produced significantly higher amounts of IFN- γ ($P < .01$). IL-10 and TGF- β production was not significantly different between splenocytes from sham-treated and FAHF-2–treated groups. There was no difference in cytokine levels between sham-treated and FAHF-2–treated groups after ConA stimulation (data not shown). These results demonstrated that FAHF-2 had a potent inhibitory effect on peanut-induced T_H2 responses throughout the 5-week posttherapy period. Enhancement of IFN- γ production was delayed posttherapy. There were minimal, if any, effects on IL-10 and TGF- β production.

Safety of FAHF-2

In a preliminary assessment of safety, we tested FAHF-2 for lethality (LD50 test). No mouse died within 12 hours after feeding 12 times the effective mouse daily dose of FAHF-2 ($n = 10$), nor did any die during the following week. In a second set of experiments, we fed 10 mice 24 times the effective mouse daily dose of FAHF-2, the maximal dose that we can feed to the mice. No mouse died

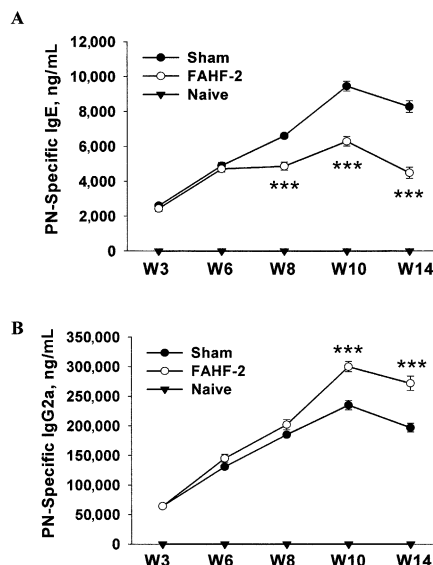


FIG 3. Effect of FAHF-2 on serum peanut-specific IgE and IgG2a levels. Sera were obtained 1 day before treatment (week 3), at each boosting (weeks 6 and 8), and 1 and 5 weeks posttherapy (weeks 10 and 14) in week 14 challenge experiments. Serum peanut-specific IgE (A) and IgG2a (B) levels were determined by ELISA. Data are means $\pm$ SEMs for each group from 3 sets of week 14 challenge experiments (sham, $n = 21$; FAHF-2, $n = 13$; naive, $n = 15$). *** $P < .001$ vs sham.

within the 2 weeks after the dose, and all mice appeared healthy.

To assess safety further, specimens from the mice tested were subjected to biochemical analysis of BUN and ALT for liver and kidney functions, respectively, 2 weeks after feeding. All results were within the normal range (Table III). In addition, CBC testing results were all in the normal range. White blood cells, red blood cells, hemoglobin, and platelets are shown in Table III. All of the major organs analyzed appeared normal (data not shown). Taken together, these results demonstrated that FAHF-2 has no obvious toxicity, even at doses well above the therapeutically effective dose.

DISCUSSION

Peanut allergy is potentially life-threatening, and there is no curative treatment. Although several novel immunotherapeutic approaches reported previously showed some degree of suppression of PNA, complete protection against peanut-induced severe systemic anaphylaxis has not been established.^{7,11,29,30} We report that FAHF-2 treatment in a murine model completely blocked anaphylaxis after peanut challenge for as long as 5 weeks posttherapy. Complete protection was confirmed by using several well-established parameters, including anaphylactic symptom scores, decreased core body temperatures, elevated plasma histamine levels,¹⁴ and vascular leakage,²⁶ and was reproducible in a series of 5 experiments at

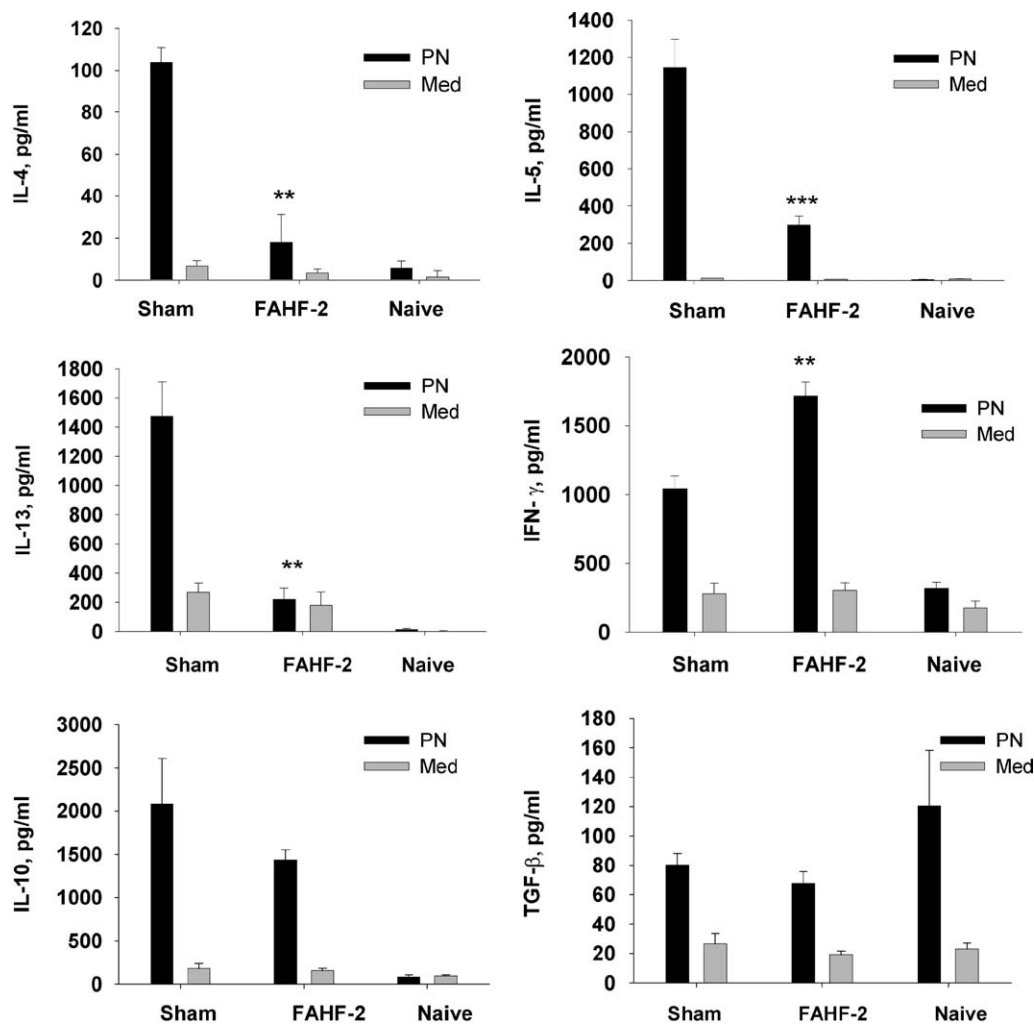


FIG 4. Cytokine levels in splenocyte culture supernatants. Splenocytes were pooled from each group after week 14 challenge. Results are expressed as means $\pm$ SEMs of cytokines for 3 sets of week 14 challenge experiments. ** $P < .01$ and *** $P < .001$ vs sham. PN, Crude peanut extract; Med, medium.

TABLE III. Biochemical assays and CBC testing*

	Biochemical assays		CBC testing			
	BUN (mg/dL)	Alanine aminotransferase (U/L)	White blood cells ($10^3/\mu\text{L}$)	Red blood cells ($10^6/\mu\text{L}$)	Hemoglobin (g/dL)	Platelets ($10^3/\mu\text{L}$)
FAHF-2	21.6 $\pm$ 2.41	39.6 $\pm$ 5.86	10.92 $\pm$ 1.23	9.05 $\pm$ 0.54	13.56 $\pm$ 0.90	732.4 $\pm$ 144.6
Sham	17.8 $\pm$ 2.17	31.8 $\pm$ 4.60	7.42 $\pm$ 2.90	8.26 $\pm$ 0.29	13.8 $\pm$ 0.8	873.8 $\pm$ 117.8
Reference	9-36	22-400	1.8-10.7	6.36-9.42	11.0-15.1	592-2972

*Mice were euthanized with CO₂, and blood was collected by cardiac puncture. Biochemical assays and CBC testing were conducted as described in Methods. Results are means $\pm$ SDs of 5 mice from each group.

different challenge times posttherapy, demonstrating a potent and a consistent effect of FAHF-2 therapy on PNA. FAHF-2 protection against peanut anaphylaxis lasted for at least 5 weeks posttherapy, suggesting that FAHF-2 may have quite a long-lasting protective effect. Furthermore, this formula appears to have a large margin of safety. These results indicated that FAHF-2, or certain components in it, have therapeutic potential for treating

PNA. Further studies are needed to identify the specific effective herbs in the mixture, first in mice and then in human subjects. Because reduced peanut-specific IgE remained significantly lower 5 weeks posttherapy, FAHF-2 may prove to be more advantageous than anti-IgE treatment, because free-IgE increased as early as 2 weeks after discontinuation of treatment with the humanized monoclonal anti-IgE antibody (rhUmAb-E25).³¹

Although peanut-specific IgE was not eliminated at any time point, there was complete protection against peanut-induced anaphylaxis. One possibility is that IgE levels in FAHF-2-treated mice were below the threshold required to trigger anaphylaxis. IgG1 can also be involved in anaphylaxis in mice³²; however, it is not associated with PNA in this model.⁹ In this study, we also found that there was no association between IgG1 levels and clinical protection by FAHF-2. IgG2a, a T_H1 driven antibody³³ is generally considered to be a blocking antibody involved in immunotherapy.³⁴ We found that IgG2a levels were significantly increased in FAHF-2-treated mice as long as 5 weeks posttherapy. Although FAHF-2 enhancement of IgG2a occurred later than its reduction of IgE, the maintained increased IgG2a levels may also play a role in producing a persistent effect of FAHF-2 on PNA posttherapy.

Numerous studies have demonstrated that T_H2 cytokines play a central role in the pathogenesis of allergic disorders, including food allergy. IL-4/IL-13 promotes B-cell switching to IgE production and mast cell activation. IL-5 has a potential autocrine effect on mast cells in addition to its recognized paracrine effects on eosinophils.^{35,36} Turcanu et al²⁸ showed that peanut allergy is dependent on T_H2 response characterized by low IFN- γ and elevated IL-4 compared with that in peanut-tolerant individuals. Thus, downregulation of T_H2 responses might be a fundamental immunotherapeutic mechanism underlying FAHF-2 effects on PNA. This hypothesis is supported by the findings in this study that FAHF-2 significantly suppressed the key T_H2 cytokines, IL-4, IL-5, and IL-13, for at least 5 weeks posttherapy. Interestingly, we also found that FAHF-2 increased IFN- γ production. However, because increased IFN- γ level, like IgG2a, was of late onset, FAHF-2 therapy-induced T_H1 response might be a secondary response to the persistent suppression of T_H2 cytokines. It has been suggested that the human intestinal system preferentially responds with a dominant T_H1 profile against various food antigens in the normal state,³⁷ and that the strong bias toward T_H1 cell responses in the human gut contributes to the fact that the majority of children with food allergy outgrow their problem.³⁸ A previous study showed that the T_H1-inducing cytokine IL-12 significantly suppressed peanut hypersensitivity reactions.²⁹ Therefore, increased IFN- γ may be an additional beneficial effect for PNA treatment because it could in turn suppress T_H2 responses and thereby re-establish stable cytokine homeostasis favorable to oral peanut tolerance. Recently, TGF- β and IL-10, which downregulate T_H1-mediated autoimmune responses, have also been suggested to play a role in allergen immunotherapy.³⁹ However, the role of IL-10 and TGF- β in food allergy has not been well established. We found no significant differences in TGF- β or IL-10 production by splenocytes from FAHF-2-treated and sham-treated mice in this study. FAHF-2 suppression of T_H2 cytokine secretion may be a result of direct effects on T_H2 cells. We recently found that the Chinese herbal formula MSSM-002 directly suppressed polarized T_H2

cell cytokine secretion, independent of IFN- γ , by suppressing the T_H2 transcription factor GATA-3.⁴⁰ Further research is needed to confirm these possibilities.

In conclusion, we demonstrated in the current study that FAHF-2, an improved herbal formula, completely blocked peanut-induced anaphylaxis for as long as 5 weeks posttherapy. This complete protection by FAHF-2 was reproducible across multiple experiments, showing an efficacious, consistent, and relatively persistent effect of FAHF-2 on PNA. This effect was associated with suppression of peanut-specific IgE, which might be secondary to downregulation of T_H2 responses. These results indicate that FAHF-2 may prove to be an effective and safe herbal therapy for PNA. Studies are underway to investigate whether similar protection occurs when the treatment regimen is initiated after more long-standing peanut hypersensitivity, ie, after at least 1 peanut challenge; to investigate how long FAHF-2 protection lasts after the initial challenge by rechallenging the mice on a monthly basis; and to investigate the actions of individual herbs in FAHF-2 on peanut allergy by using the same model.

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