

Food Allergy Herbal Formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in a murine model

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Background: Peanut allergy is a major cause of fatal and near-fatal anaphylactic reactions to foods. There is no curative therapy for this condition. Traditional Chinese medicines have been reported to have antiallergic properties, which might be useful for treating peanut allergy.

Objective: The purpose of this study was to investigate the effects of a Chinese herbal formula, FAHF-1, on peanut anaphylactic reactions in a mouse model of peanut allergy.

Methods: Mice were sensitized with freshly ground whole peanut in the presence of cholera toxin and boosted 1 and 3 weeks later. FAHF-1 treatment was initiated 1 week later and continued for 7 weeks. After treatment, mice were challenged with peanut, and anaphylactic symptoms, body temperatures, and plasma histamine and IgE levels were measured. T-cell proliferative responses and cytokine production were also determined.

Results: FAHF-1 completely blocked peanut-induced anaphylactic symptoms and markedly reduced mast cell degranulation and histamine release. Peanut-specific serum IgE levels were significantly reduced by 2 weeks of treatment at the time of challenge, and they remained lower 4 weeks after discontinuation of treatment. FAHF-1 significantly reduced peanut-induced lymphocyte proliferation as well as IL-4, IL-5, and IL-13 synthesis but not IFN- γ synthesis. No toxic effects on liver or kidney functions were observed, nor was there any overall immune suppression.

Conclusion: FAHF-1 protected peanut-sensitized mice from anaphylactic reactions and significantly reversed established IgE-mediated peanut allergy. This suggests that FAHF-1 might prove valuable for the treatment of peanut allergy. (*J Allergy Clin Immunol* 2001;108:639-46.)

Key words: Peanut anaphylaxis, traditional Chinese medicine, IgE, mast cells, T_H2 cytokines

Abbreviations used

Con A: Concanavalin A

FAHF-1: Food Allergy Herbal Formula-1

PN: Peanut

TCM: Traditional Chinese medicine

Food allergy currently affects approximately 2% of the US population and appears to be increasing.¹ Approximately 1.5 million Americans are allergic to peanut (PN),² and anaphylactic reactions to PN account for approximately 15,000 emergency room visits per year.³ In contrast to other food allergies, such as milk and egg allergy, most patients do not “outgrow” PN allergy.^{4,5}

At the present time there are no effective therapies for IgE-mediated food allergies, and patients must be instructed in food-allergen avoidance and self-treatment after accidental ingestion. However, accidental ingestion of PN-containing food is not rare and results in many deaths each year.⁶ The apparently increasing prevalence of PN allergy and the “lifelong” nature of this sensitivity increases the urgency for the development of effective therapies.

Traditional Chinese medicine (TCM), used in Asia for centuries, is attracting interest in Western countries as a source of alternative or complementary therapies for a variety of diseases, including asthma.^{7,8} We recently reported that the herbal formula MSSM-02 reversed allergic airway hypersensitivity associated with reduction of T_H2 responses in a mouse model of allergic asthma.⁹ This prompted us to investigate the effect of TCM herbal intervention for the treatment of PN allergy. Unlike asthma and other allergic diseases, food allergy is not described in the TCM literature, and no previous research into developing herbal interventions for food allergy has been reported. In the light of the gastrointestinal symptoms induced by food allergic reactions and the T_H2-dominant responses of food allergy, we hypothesized that a TCM herbal formula, Food Allergy Herbal Formula-1 (FAHF-1) containing *ling zhi*, an herb shown to have “anti-inflammatory” and antiallergy properties,^{10,11} and *wu mei wan*, which is used to treat colic, vomiting, and chronic diarrhea accompanying ascaris infestation, might be useful for the treatment of food allergy. In the present study, we tested the therapeutic effects of this formula in a murine model of PN allergy.¹² We found that FAHF-1 blocked systemic anaphylactic

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A US Provisional Patent Application (reference number 2002834-0114) regarding FAHF-1 has been filed.

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TABLE I. Components of herbal medicines in FAHF-1

	TCM materia medica (pinyin)*	Equivalent pharmaceutical name*	Amount (g)	Part used
1	<i>Ling zhi (Chi)</i>	Ganoderma lucidum	15	Fruiting body
2	<i>Fu zi (zhi)</i>	Radix lateralis aconiti carmichaeli praeparata	3	Root
3	<i>Wu mei</i>	Fructus pruni mume	30	Fruit
4	<i>Chuan jiao</i>	Pericarpium zanthoxyli bungeani	1.5	Seed
5	<i>Xi xin</i>	Herba cum radice asari	1	Whole
6	<i>Huang lian (Chuan)</i>	Rhizoma coptidis	9	Root
7	<i>Huang bai</i>	Cortex phellodendri	6	Root
8	<i>Gan jiang</i>	Rhizoma zingiberis officinalis	6	Root
9	<i>Gui zhi</i>	Ramulus cinnamomi cassiae	3	Twig
10	<i>Ren shen (Hong)</i>	Radix ginseng	9	Root
11	<i>Dang gui (shen)</i>	Corpus radix angelicae sinensis	9	Root
Total			92.5	

References: Huang³⁰; Bensky and Gamble.³¹

symptoms after PN challenge of allergic mice, no toxic effects on liver or kidney functions and no overall immune suppression being observed. These results suggest that FAHF-1 might be useful for the treatment of food allergy.

MATERIALS AND METHODS

Mice and reagents

Five-week-old female C3H/HeJ mice purchased from the Jackson Laboratory (Bar Harbor, Me) were maintained on PN-free chow under specific pathogen-free conditions. Standard guidelines¹³ for the care and use of animals were followed.

Freshly ground, roasted, whole PN was employed as allergen. Crude PN extract was prepared as described previously.^{14,15} Cholera toxin was purchased from List Biological Laboratories, Inc (Campbell, Calif). Concanavalin A (Con A) and albumin, human-dinitrophenyl (DNP-albumin) were purchased from Sigma (St Louis, Mo). Antibodies for ELISAs were purchased from The Binding Site, Inc, (San Diego, Calif) or PharMingen (San Diego, Calif). All of the herbal medicines (which were all of Chinese origin) were inspected by pharmacists trained and licensed in identification and processing of herbal medicines according to the Chinese Herbal Medicine Materia Medica and Pharmacopoeia of the People's Republic of China at the China-Japan Friendship Hospital, Beijing, China.

FAHF-1 preparation

FAHF-1 consists of 11 herbs (Table I). The daily human adult dosage (95.5 g; Table I) of this formula was decocted (water-extracted) as described by Bensky and Barolet.¹⁶ Briefly, component 1, *ling zhi*, was boiled separately for 2 hours, and the decoction was filtered and lyophilized (extract A). Component 2, *fu zi*, was boiled for 2 hours, and the remaining components were then added and boiled for an additional 1 hour. The resulting decoction was lyophilized (extract B) and mixed with extract A, the result being 15 g of dried extract. Through use of a conversion table of equivalent effective dose ratios from human beings to animals based on body surface area,¹⁷ 21 mg per mouse of lyophilized decoction in 0.5 mL was administered to each mouse twice daily.

PN sensitization/challenge and FAHF-1 treatment

Mice were sensitized with PN (5 mg per mouse) plus cholera toxin (10 µg per mouse), administered intragastrically and boosted

1 and 3 weeks later. One week after the final sensitization dose, mice received either 21 mg of FAHF-1 or water (sham) treatment by intragastric gavage twice daily for 7 weeks. Mice were then challenged intragastrically with 10 mg of crude PN extract. Naive mice served as additional controls.

Assessment of hypersensitivity reactions

Type I hypersensitivity reactions were scored as previously described:¹²

- 0, no symptoms
- 1, scratching and rubbing around the nose and head
- 2, puffiness around the eyes and mouth, pilar erecti, diarrhea, and reduced activity or standing still with an increased respiratory rate
- 3, wheezing, labored respiration, and cyanosis around the mouth
- 4, symptoms as in no. 3 with loss of consciousness, tremors, and/or convulsion
- 5, death.

Measurement of plasma histamine levels and mast cell degranulation

Plasma was collected 30 minutes after challenge, and histamine levels were determined through use of an enzyme immunoassay kit (ImmunoTECH, Marseille, France), as previously described.¹² The numbers of degranulated mast cells in ear tissues collected 40 minutes after the PN challenge were determined as previously described.¹⁸

Measurement of serum antibodies

PN-specific serum IgE concentrations were measured by ELISA, as described previously.¹² For measurement of total serum IgG and IgA, 96-well plates were coated with rat antimouse IgG or IgA capture antibody (2 µg/mL) in coating buffer, pH 9.6. Plates were then blocked and washed in the manner described above. Samples (1:50,000 dilution for total IgG and 1:500 dilution for total IgA) and 10 serial 1:2 dilutions of mouse IgG or IgA (standard curve) were added to the plates and incubated overnight at 4°C. After appropriate washing, biotinylated rat antimouse IgG (0.1 µg/mL) or IgA (2 µg/mL) mAbs were added to the plates and incubated for an additional 1 hour at room temperature. After further washings, avidin peroxidase was added and the plates were washed 15 minutes later. The reactions were developed with ABTS (KPL, Gaithersburg, Md) for 30 minutes at room temperature and read at 405 nm.

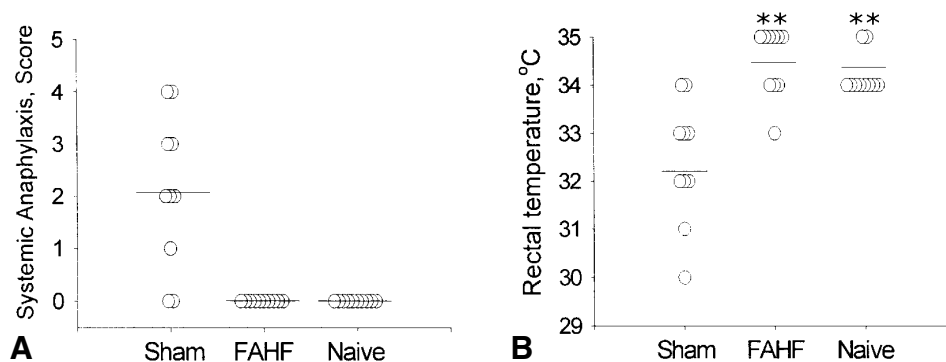


FIG 1. Protection against PN-induced anaphylactic reactions by FAHF-1. **A**, Anaphylactic symptom scoring was done 30 to 40 minutes after the last challenge dose, as described in the Materials and Methods section. Symbols (○) indicate individual mice from 2 sets of experiments ($n = 10$). Bars are medians of scores. **B**, Rectal temperatures were measured 20 minutes after PN challenge by intragastric gavage. Symbols (○) indicate individual mice from 2 sets of experiments ($n = 10$). Bars are means of temperatures. $**P < .01$ vs sham.

T-cell culture, proliferation assays, and quantitation of cytokines

Splenocytes from 5 mice in each group were incubated in triplicate cultures in the presence or absence of crude PN extract (50 $\mu\text{g/mL}$), as described previously.¹² Cells stimulated with Con A (2 $\mu\text{g/mL}$) served as a positive control. Three days later, the cultures were pulsed for 18 hours with 1 μCi per well of ^3H -thymidine. Cells were then harvested, and the incorporated radioactivity was determined in a β scintillation counter. Results are expressed as counts per minute.

Splenocytes were cultured in 24-well plates (4×10^6 /well/mL) in the presence or absence of crude PN extract (50 $\mu\text{g/mL}$) or Con A (2 $\mu\text{g/mL}$). Supernatants were collected 72 hours later. IL-4, IL-5, IL-13, and IFN- γ were determined by ELISA, according to the manufacturer's instructions.

Toxicity testing

Sera from FAHF-1-treated PN-allergic mice were obtained 1 day before challenge and subjected to biochemical analyses of liver and kidney function testing.

To further evaluate possible toxicity, naive mice were fed twice the therapeutic dose of FAHF-1 (42 mg/mouse) for 7 weeks (the therapeutic treatment course) and 14 weeks (twice the therapeutic treatment course) in 3 separate experiments. Sera were obtained from the mice after 7 and 14 weeks of treatment and 7 weeks after discontinuation of treatment. Liver and kidney functions were tested through use of PROCHEM-V instrumentation (Synbiotics, San Diego, Calif). Complete blood counts were determined. Histologic analyses of organs (liver, kidney, heart, spleen, lung, intestines, and stomach) were performed.

To determine whether FAHF-1 had toxic or generalized immune-suppressive effects on B and T cells, total serum IgG and IgA levels were measured. Splenocytes were cultured in the presence or absence of Con A, and IFN- γ levels were determined by ELISA.

Statistical analysis

Data were analyzed through use of a SigmaStat 2.03 statistical software package (SPSS Inc, Chicago, Ill). Statistical differences in IgE levels, percent mast cell degranulation, and T-cell proliferation between 2 groups (FAHF-1-treated and sham-treated) were compared through use of a Student t test if they were determined to be normally distributed. A Mann-Whitney rank sum test was used to analyze comparisons of in vitro cytokine secretion between the 2 groups. For rectal temperatures, the differences between 3 groups

(FAHF-1-treated, sham-treated, and naive) were analyzed by ANOVA and then by a Bonferroni t test for all pairwise comparisons. P values less than .05 were considered statistically significant.

RESULTS

Protection from PN-induced anaphylactic reactions

Anaphylactic symptom scores were determined 30 to 40 minutes after oral PN challenge. As depicted in Fig 1, A, 80% of the mice in the sham-treated group exhibited symptoms such as itching (score 1, 10%); puffiness around the eyes, swelling around the mouth, and diarrhea (score 2, 30%); labored respiration (score 3, 20%); and loss of consciousness or little activity after prodding (score 4, 20%). The median score was 2.2. In contrast, no symptoms were observed in the FAHF-1-treated mice or in the naive mice after challenge (Fig 1, A). Because a drop in core body temperature reflects the severity of systemic anaphylaxis in mice,¹⁹ rectal temperatures were obtained 30 minutes after the PN challenge. Temperatures in the naive group ranged between 34° and 35°C after oral PN challenge, whereas rectal temperatures of 8 of 10 mice in the sham-treated group were 1° to 4°C below normal. In contrast, only 1 of 10 mice in the FAHF-1-treated group had a decrease in core temperature of 1°C. There was a significant difference in mean temperature between the sham-treated group and the FAHF-1-treated group ($P < .001$) but not between the FAHF-1-treated group and the naive group (Fig 1, B). These results demonstrate that FAHF-1 protected PN-allergic mice from PN-induced anaphylactic reactions.

Reduction of mast cell degranulation and plasma histamine levels

Mast cell degranulation and histamine release are major factors in anaphylaxis. Consistent with our previous findings,²⁰ numerous degranulated mast cells were observed in tissues of the sham-treated mice challenged with PN (Fig 2, A and B). The numbers of degranulated mast cells were

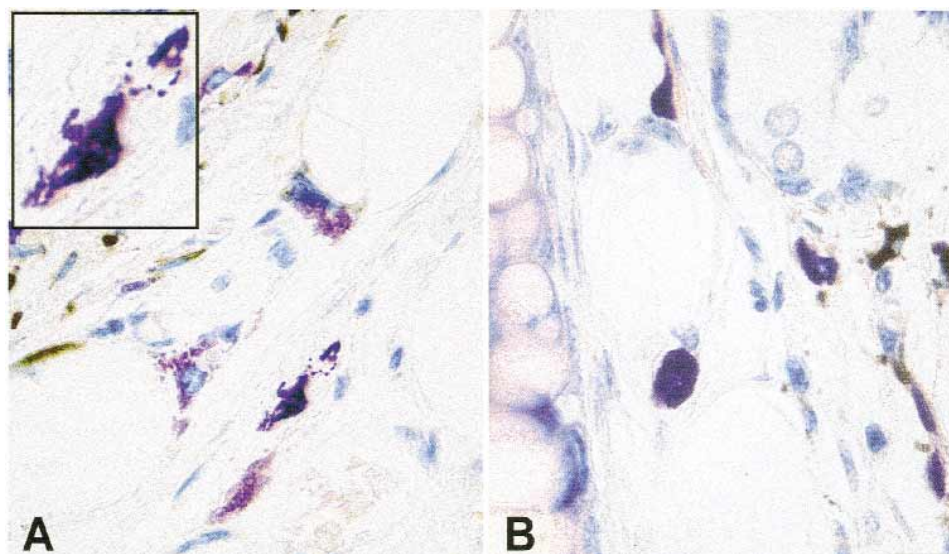


FIG 2. Degranulation of mast cells. Five-micrometer paraffin sections of ear samples were collected for 40 minutes; paraffin sections were stained with toluidine blue and examined by means of light microscopy. **A**, Degranulated mast cells in ear samples of sham-treated mice after challenge (bar represents 10 μ m). *Inset*: High magnification shows granules outside degranulating mast cells. **B**, Normal mast cells in ear sample from FAHF-1-treated mouse.

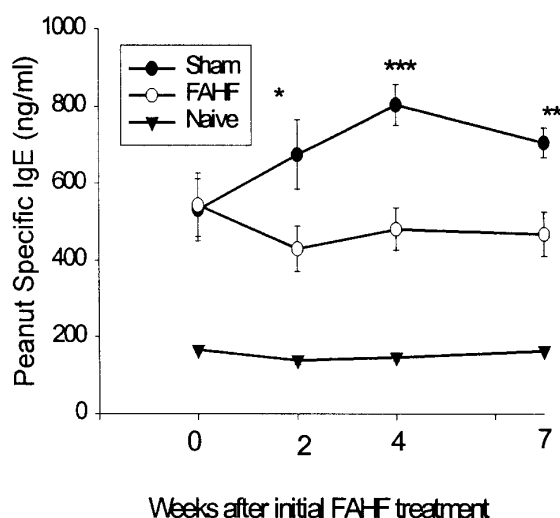


FIG 3. Levels of PN-specific IgE after initiation of FAHF-1 treatment. Sera from all groups of mice were obtained 4 weeks after the initial sensitization, at which time FAHF-1 treatment was initiated (week 0). Blood samples were obtained every 2 to 3 weeks during the treatment for 7 weeks. PN-specific IgE levels were determined by ELISA. Data are given as means $\pm$ SEMs for each group (sham, $n = 10$; FAHF-1, $n = 10$; naive, $n = 5$) from 2 experiments. * $P < .05$ vs sham. ** $P < .01$ vs sham. *** $P < .001$ vs sham.

significantly lower in the FAHF-1-treated mice than in the sham-treated mice ($50.6\% \pm 4.7\%$ in the sham group vs $20\% \pm 4\%$ in the FAHF-1 group; $P < .001$) and were not significantly different from those seen in normal controls ($13\% \pm 2\%$). Plasma histamine levels also were markedly elevated in the sham-treated group (5414 ± 3802 nmol/L) but not in the FAHF-1-treated group (52 ± 31 nmol/L) in

comparison with those in naive mice (29 ± 11 nmol/L). These results demonstrated that FAHF-1 treatment of PN allergic mice largely blocked PN-triggered mast cell degranulation and histamine release.

Reduction of PN-specific IgE levels

FAHF-1 treatment was initiated 4 weeks after PN sensitization, at which time PN-specific IgE levels were markedly elevated in both sensitized groups (mean $\pm$ SEM, 531 ± 80 ng/mL in the FAHF-1 group vs 544 ± 82 ng/mL in the sham group; Fig 3, A). After 2 weeks of treatment, IgE levels in the FAHF-1-treated group were significantly lower than those in the sham-treated group and remained significantly lower throughout the course of treatment. Additional mice were not killed after challenge, treatment was discontinued, and IgE levels were monitored for an additional 4 weeks. At that time, IgE levels were essentially the same as they had been when treatment was discontinued (347 ± 98 at week 0, 343 ± 69 at week 4), and were approximately one half as great as the levels seen in sham-treated mice (726 ± 95 ng/mL), demonstrating that FAHF-1 had a persistent effect lasting for at least 4 weeks. In addition, total IgE and IgG levels in the FAHF-1-treated group were not significantly different from those in the sham-treated group (data not shown).

Suppression of PN-specific T-cell proliferation and T_H2 cytokine production

Consistent with our previous finding, splenocytes from sham-treated, PN-allergic mice showed a marked in vitro proliferative response to PN stimulation (Fig 4, A). In contrast, the proliferative response of splenocytes from FAHF-1-treated, PN-allergic mice after PN stimu-

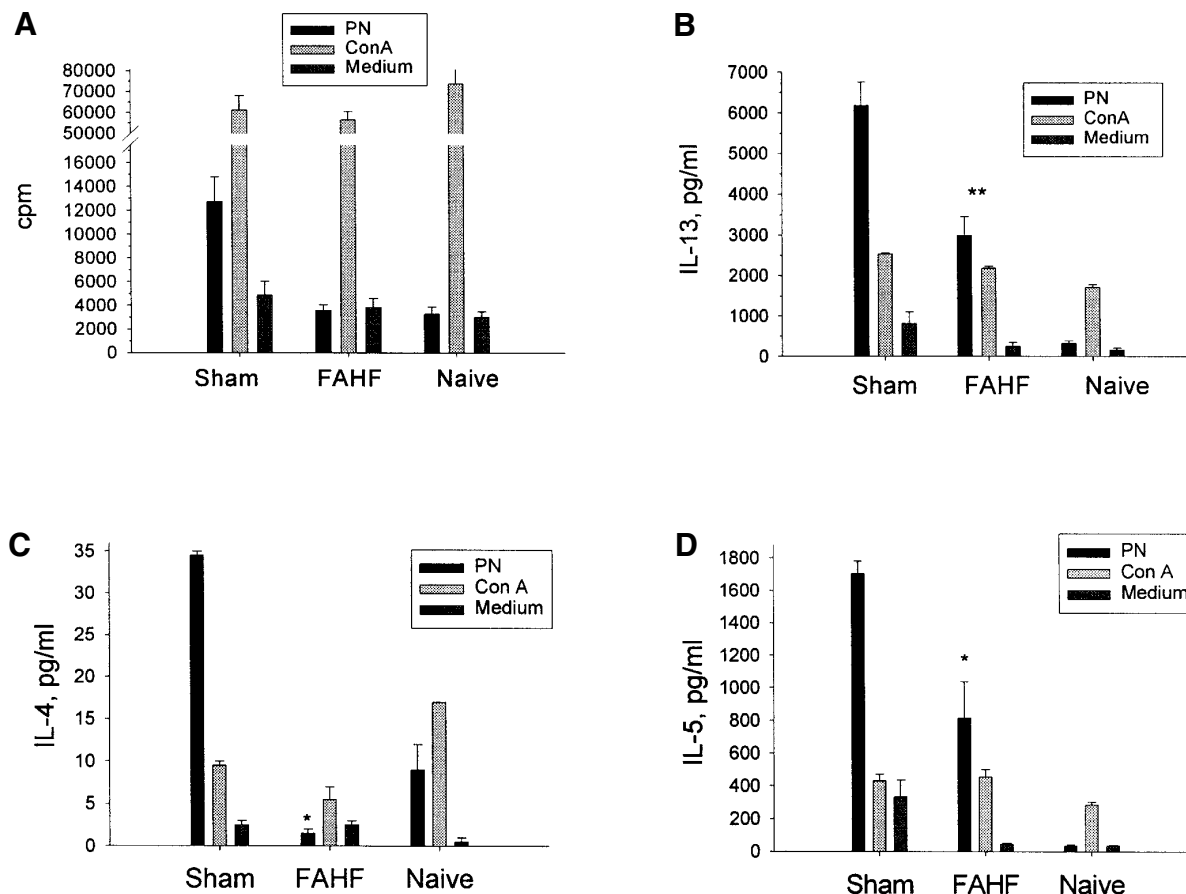


FIG 4. A, Splenocyte proliferative responses. Immediately after evaluation of anaphylactic reactions, mice were killed and spleen cells were isolated from each group of mice and pooled ($n = 5$). Cells were cultured with crude PN extract, Con A, or medium alone. Three days later, the cultures were pulsed for 18 hours with 1 μ Ci per well of ^{3} H-thymidine. The cells were harvested and the incorporated radioactivity was determined. Data are presented as means $\pm$ SEMs of counts per minute from 2 sets of triplicate wells of 2 experiments. $**P < .01$ vs sham. B, C, D, Cytokine levels. Cell suspensions were cultured in complete culture medium in the presence of PN antigen, Con A, or medium alone. Supernatants were collected 72 hours later, and IL-4, IL-5, and IL-13 were determined by ELISA. Results are expressed as means $\pm$ SEMs of 2 duplicate cultures from 2 experiments. $*P < .05$. $**P < .01$ vs sham.

lation was significantly less than that of splenocytes from sham-treated mice ($P < .01$), being essentially the same as that of splenocytes from naive spleen cells. Interestingly, FAHF-1 treatment did not decrease the proliferative response to Con A stimulation.

Splenocytes from FAHF-1-treated mice secreted significantly less IL-4, IL-5, and IL-13 than splenocytes from sham-treated mice after PN stimulation (Fig 4, B through D). However, IL-4, IL-5, and IL-13 secretion levels after Con A stimulation of splenocytes from FAHF-1-treated and sham-treated mice were not significantly different. FAHF-1 treatment did not alter either PN- or Con A-stimulated IFN- γ production (for PN, 1367 ± 582 ng/mL in the sham group and 1417 ± 1140 ng/mL in the FAHF-1 group; for Con A, 1461 ± 569 ng/mL in the sham group and 1761 ± 1132 ng/mL in the FAHF-1 group). These results suggest that FAHF-1 treatment resulted in specific suppression of both PN-induced T-cell proliferation and PN-activated T_H2 cytokine secretion.

Oral FAHF-1 treatment had no detectable toxic effects

Results of liver and kidney function tests of PN-allergic and naive mice treated with the therapeutic dose, as well as of mice treated with twice the therapeutic dose, of FAHF-1 for 7 and 14 weeks were within the normal reference values and not different from those of untreated naive or sham-treated PN-allergic mice (Table II). Hemoglobin levels, numbers of red and white blood cells, and differentials were all in the normal range (data not shown). No gross or histologic abnormalities of major organs (liver, kidney, heart, spleen, lung, intestines, stomach) were observed in any group. Body weights of FAHF-1-treated mice did not differ from those of untreated mice (data not shown). No deaths or evidence of poor health were observed in FAHF-1-treated mice.

Total serum IgG and IgA levels in naive mice after 7 weeks of FAHF-1 treatment were slightly but not signif-

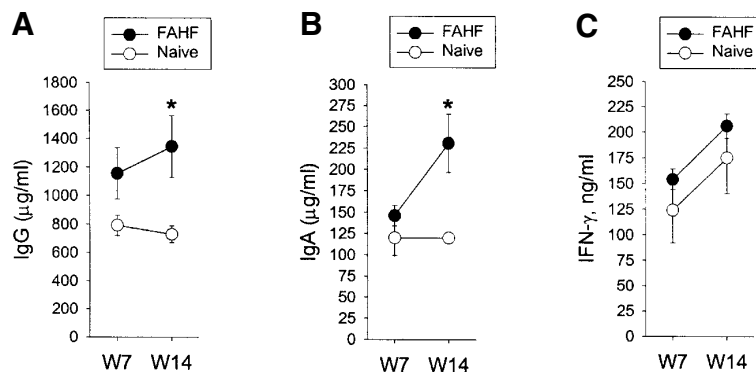


FIG 5. A, B, Total serum IgG and IgA. Naive mice were treated with FAHF-1 ($n = 10$) or water ($n = 10$) for 7 and 14 weeks. Sera were obtained, and total IgG (A) and IgA (B) levels were determined by ELISA. Results are expressed as means $\pm$ SEMs. * $P < .05$ vs naive. **C,** Levels of IFN- γ . Splenocytes from the same mice were cultured in the presence or absence of Con A. Supernatants were harvested 72 hours later. Levels of IFN- γ were determined by ELISA.

TABLE II. Biochemical assays of liver and kidney functions

	Naive/FAHF*	Naive/FAHF†	PN/FAHF‡	PN/sham‡	Naive/FAHF§	Naive	Reference range	Unit
BUN	14.8	17.6	20	22	18	15	9-36	mg/dL
Total protein	5.5	4.6	5.8	5.8	5.2	4.2	5.9-6.9	g/dL
Albumin	3.4	3.2	3.2	3.5	2.6	3.2	2.4-4.4	g/dL
Creatinine	0.5	0.4	0.6	0.7	0.3	0.4	0.4-1	mg/dL
T-bilirubin	0.6	0.4	0.8	0.8	0.3	0.3	0.2-0.5	mg/dL
ALT (SGPT)	40.3	47	36	36	40	41	22-400	U/L
Alb/Glob ratio	1	1.5	1.3	1.5	1	2.5	0.6-1.2	—
Globulin	3.5	2.1	2.5	2.3	2.5	1	2.1-4.3	g/dL

Alb/Glob, Albumin/globulin.

*Naive mice were fed a double dose of FAHF-1 (42 mg/mouse, twice daily) for 7 weeks ($n = 10$).

†Naive mice were fed a double dose of FAHF-1 (42 mg/mouse, twice daily) for 14 weeks ($n = 5$).

‡PN-allergic mice were treated with FAHF-1 (21 mg/mouse, twice daily) or PBS for 7 weeks ($N = 10$).

§Naive mice were fed a double dose of FAHF-1 (42 mg/mouse, twice daily) for 7 weeks ($n = 10$), and blood was drawn 7 weeks after discontinuation of the treatment.

icantly higher than those in untreated naive mice; interestingly, after 14 weeks of FAHF-1 treatment, total serum IgG and IgA levels were significantly higher than those in untreated mice ($P < .05$; Fig 5). IFN- γ levels slightly but not significantly increased in FAHF-1-treated naive mice at both week 7 and week 14 in comparison with the levels in untreated mice. These results demonstrated that oral FAHF-1 treatment had no detectable toxic effects or overall immune suppression.

DISCUSSION

In PN-allergic individuals, exposure to PN proteins induces IgE-mediated activation of mast cells/basophils, resulting in the release of histamine and other mediators, which provoke anaphylactic symptoms involving multiple organs, including the skin, gastrointestinal tract, and respiratory system. In the most severe cases, the cardiovascular system is also involved and systemic shock develops. In this study, we found that a novel herbal formula, FAHF-1, eliminated all symptoms of anaphylaxis and markedly reduced mast cell degranulation and histamine release in a murine model of PN allergy. Furthermore, 2 weeks of

treatment significantly reduced serum IgE levels, which remained lower for at least 4 weeks after discontinuation of treatment. These results demonstrated that FAHF-1 largely reversed established PN allergy and protected sensitized mice from anaphylactic reactions after PN challenge. Further studies will be required to determine the duration of FAHF-1-induced IgE suppression and the effects of intermittent treatment regimens.

Because classic immunotherapy is not an option for PN-allergic individuals,^{21,22} several alternative therapeutic approaches are being tested. We previously found that preimmunization of mice with pAra h2 (plasmid DNA-encoding Ara h2) partially protected AKR mice from PN sensitization and challenge but resulted in anaphylaxis in C3H mice.^{20,23} Roy et al²⁴ reported that prophylactic oral administration of the same pAra h2 embedded in chitosan particles inhibited PN sensitization of AKR mice. However, there are no published reports of successful immunotherapy for "established" PN allergy. Although prophylactic protocols using cytokines such as IL-12,²⁵ IFN- γ ,²⁶ and anti-IL-4 receptor antibody²⁷ in animal models of allergic asthma have provided some basis for the use of immunoregulatory modulators in allergic

disorders, therapeutic strategies must ameliorate established allergy to be clinically relevant. We recently found that both cytosine-guanine oligodeoxynucleotides and an herbal formula, MSSM-002, administered after allergen sensitization and challenge (ie, in established allergy) were able to partially reverse T_H2 -mediated allergic airway responses.²⁸ In this study, we treated PN-sensitized mice with an herbal formulation and demonstrated that this treatment abrogated PN-induced anaphylactic symptoms and significantly reduced PN-specific serum IgE levels. Our results suggest that an immune system already "committed" to an anaphylactic pathway can be normalized, at least partially, after treatment with FAHF-1 in this model.

As they are in other allergies, T_H2 cytokines appear to be dominant in food allergies.²⁹ In this study, we found that FAHF-1 suppressed in vitro PN-induced IL-4, IL-5, and IL-13 cytokine secretion and PN-induced splenocyte proliferation without altering IFN γ production. The precise means by which FAHF-1 prevents PN-induced hypersensitivity reactions are unknown; specific downregulation of T_H2 cytokines might, at least partially, account for FAHF-1-induced suppression of PN hypersensitivity. It is also possible that FAHF-1 directly suppresses PN-induced B-cell activation, thereby reducing the number of allergen-specific IgE molecules bound to mast cells (with subsequent reduction of FC ϵ RI levels on the surface of mast cells), or that FAHF-1 directly inhibits mast cell degranulation. It is also conceivable that FAHF-1 reduces intestinal permeability, thereby reducing the amount of PN allergens available to interact with mast cells. It is possible that FAHF-1 targets multiple parts of a food allergic reaction cascade. Further studies will be required to explore the mechanisms responsible for the suppression of allergic reactions by FAHF-1.

As noted, Chinese herbal formulations are often composed of more than 10 different herbs; FAHF-1 is composed of 11. These combinations frequently exhibit synergistic effects in relieving symptoms as well as in reducing possible side effects caused by one or more of the herbs in the formula. All constituents in FAHF-1 are considered to be safe when used according to classical Chinese herbology practice. In this study, we found no evidence of in vivo toxic effects on the kidney, liver, or other organs after 14 weeks of FAHF-1 treatment. Furthermore, we found that in contrast to corticosteroids, which induce immune suppression, FAHF-1 suppressed PN-specific T_H2 responses without suppressing IFN γ secretion by splenocytes from PN-allergic mice, total serum IgG and IgA levels in naive mice, or IFN- γ production by splenocytes from naive mice.

These results suggest that FAHF-1 reduction of T_H2 responses is not due to a toxic effect on immune cells or to overall immunosuppression. The antiallergy properties of the individual herbal medicines are largely unknown. However, we found in a preliminary study that neither *ling zhi* nor *wu mei wan* alone was as effective as the combination in the FAHF-1 formula (data not shown). How the combination of individual herbs in FAHF-1 synergistically improves symptoms is an interesting and challenging area for future research.

In conclusion, we describe an herbal formula, FAHF-1, that blocked anaphylaxis in a murine model of PN hypersensitivity. This effect is associated with reduction of PN-specific IgE and mast cell degranulation and with the downregulation of T_H2 cytokine production. Although animal models are not identical to human disease and further studies regarding effects of long-term administration and/or interactions with prescription drugs are required, this study suggests that FAHF-1 might be useful for the treatment of PN allergy and perhaps of other IgE-mediated food allergies.

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