

Treatment of Asthma and Food Allergy With Herbal Interventions From Traditional Chinese Medicine

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ABSTRACT

Prevalence of asthma and allergy has increased over the past 2–3 decades in Westernized countries. Despite increased understanding of the pathogenesis of asthma and allergic diseases, control of severe asthma is still difficult. Asthma is also associated with a high prevalence of anxiety, particularly in adolescents. There is no effective treatment for food allergy. Food allergy is often associated with severe and recalcitrant eczema. Novel approaches for treatment of asthma and food allergy and comorbid conditions are urgently needed. Traditional Chinese medicine, used in Asia for centuries, is beginning to play a role in Western healthcare. There is increasing scientific evidence supporting the use of traditional Chinese medicine for asthma treatment. Since 2005, several controlled clinical studies of “antiasthma” herbal remedies have been published. Among the herbal medicines, antiasthma herbal medicine intervention is the only antiasthma traditional Chinese medicine product that is a Food and Drug Administration investigational new drug that has entered clinical trials in the United States. Research into the effects and mechanisms of action of antiasthma herbal medicine intervention in animal models is actively being pursued. Research on traditional Chinese medicine herbal medicines for treating food allergy is rare. The herbal intervention Food Allergy Herbal Formula-2 is the only Food and Drug Administration botanical investigational new drug under investigation as a multiple food allergy therapy. This review article discusses promising traditional Chinese medicine interventions for asthma, food allergy, and comorbid conditions, and explores their possible mechanisms of action. *Mt Sinai J Med* 78:697–716, 2011. © 2011 Mount Sinai School of Medicine

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Formula-2, herbal interventions, traditional Chinese medicine.

BACKGROUND

Asthma

Asthma affects approximately 25 million Americans¹ and is the leading childhood chronic disease, affecting 9.6% of American children.² The prevalence of asthma in children increased 3-fold in the past 3 decades.^{3,4} Airway hyper-reactivity (AHR) to a variety of stimuli is a characteristic feature of asthma. In atopic subjects, re-exposure to the relevant antigen triggers an immediate early-phase response, often followed by a late-phase response 6–12 hours later.^{5–7} The early-phase response is triggered by mast cell degranulation and release of mediators, such as histamine.^{8,9} The late-phase response is associated with infiltration of eosinophils.⁵ However, other cells, including neutrophils, T cells, and macrophages, are also recruited during the late-phase response. Chronic inflammation may result in airway remodeling, prominent features of which are subepithelial fibrosis, mucous cell hyperplasia/metaplasia, and increased smooth muscle mass.^{10–14} These structural changes may be responsible for the accelerated progressive decrease in lung function of some patients^{15,16} and be significant determinants of AHR.¹⁷ It has been demonstrated that memory T helper 2 (Th2) cells play a central role in the pathology of allergic asthma via releasing Th2 cytokines.¹⁸ The master Th2 transcription factor GATA-3 regulates Th2 cytokine expression.^{19–22} It is also widely accepted that abnormal airway smooth muscle (ASM) function, characterized by increased contraction and/or reduced smooth muscle relaxation, with or without airway remodeling, contributes to the excessive airway narrowing and AHR characteristic of asthmatic attacks.²³

The complexity of this chronic disease is a great challenge to developing interventions that exhibit a broad spectrum of pharmacological actions on pathogenic changes of asthma. Current standard asthma management is primarily directed toward suppressing airway inflammation with inhaled corticosteroids (ICSs) and relieving bronchoconstriction with bronchodilators. This standard therapy controls symptoms in most, but not all, asthmatics.^{24,25} Severe asthma is difficult to control.^{24–26} Most asthmatic children still suffer attacks.⁴ Relapse after therapy withdrawal is common.²⁷ Inhaled corticosteroids are generally safe and efficacious, with few side effects. However, prolonged use of ICSs, especially

at higher doses, has been accompanied by concern about both systemic and local side effects^{28,29} including osteoporosis,^{28–31} hypothalamic-pituitary-adrenal axis suppression,³² immune suppression resulting in increased susceptibility to infections such as esophageal candidiasis³³ and frequent upper respiratory tract infection in children,³⁴ development of cataracts in elderly patients,³⁵ mood changes,³⁶ and pharyngitis.³⁷ There is an urgent need to develop novel approaches for treatment of asthma.

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Food Allergy

Food allergy is a significant health problem in Westernized countries, affecting 6% of children and 3%–4% of adults in the United States.³⁸ Peanut (PN) and tree nut allergies account for the majority of fatal and near-fatal anaphylactic reactions in the United States.^{39,40} The prevalence of childhood peanut allergy (PNA) has tripled since 1997. The prevalence of PN and tree nut allergy in children aged <18 years is 2.1%.⁴¹ The reasons for the rise in food allergy are unknown, but there are several hypotheses, including the “hygiene hypothesis,”⁴² the overall allergy march,⁴³ and epigenetics.^{42,44–46}

Although the precise mechanisms involved in the development of PNA have not been fully elucidated, several mechanisms involved in PNA have been identified in humans and in animal models. In genetically predisposed persons, ingestion of PN allergen induces predominant Th2 responses partially due to inadequate Th1 and/or regulatory T cell (Treg) cross-control of Th2 responses. Th2 cytokines such as interleukin-4 (IL-4) and IL-13 promote B-cell activation, switching to immunoglobulin E (IgE) production. These IgE antibodies then bind to the high-affinity Fc epsilon receptor (FcεRI) on basophils and mast cells. In patients with food hypersensitivity, re-exposure to the relevant foods triggers degranulation of mast cells and basophils, resulting in the release of histamine and other mediators (such as platelet-activating factor), which provoke symptoms of anaphylaxis. Thereafter, if there is no further

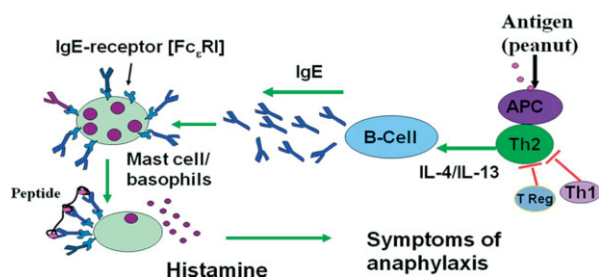


Fig 1. Immunopathogenesis of food allergy. This figure outlines some of the important aspects of the allergic cascade in PNA. Green arrows indicate positive regulation and red arrows indicate negative regulation. **Abbreviations:** APC, antigen presenting cells; FcεRI, Fc ε receptor; IgE, immunoglobulin E; IL, interleukin; PNA, peanut allergy; Th1, T helper cell type 1; Th2, T helper cell type 2; T Reg, regulatory T cells.

allergen exposure, some T cells and B cells will undergo apoptosis, but others will become long-lived memory cells that might be the important driving force for the persistent food allergy (Figure 1).

The signs and symptoms of an immediate reaction to food occur within minutes to 2 hours after ingestion. The symptoms and severity vary depending upon the routes and extent of mast cell degranulation. Mast cells are abundant in the skin, lungs, and gastrointestinal (GI) tract. In general, symptoms may be limited to the GI tract (ie, GI anaphylaxis manifested by vomiting, diarrhea, and abdominal pain). In other cases, the spread of allergen through the bloodstream leads to degranulation of mast cells in the skin, which is manifested by hives, swelling, and pruritus; or in the airway, manifested by choking, coughing, wheezing, and difficulty breathing. In the most severe cases, the cardiovascular system is also involved, with a subsequent anaphylactic shock response.

At present, the primary treatments for PNA remain PN avoidance and appropriate use of rescue medication. In 1997, Nelson *et al.*⁴⁷ investigated conventional allergen-specific immunotherapy and concluded that this therapy is not satisfactory because of the unacceptable number of adverse reactions and poor maintenance of tolerance afterward.^{47,48} Since 2009, several studies^{49–51} reported results of a newer immunotherapy strategy, oral immunotherapy (OIT) for PNA. Peanut OIT appeared to increase the patients' threshold to PN protein while on daily immunotherapy (desensitization),^{49,51} but there is, as yet, no evidence that the protection persists post-therapy (tolerance induction). Peanut OIT also causes allergic reactions,^{49,51,52} although less severe than subcutaneous PN immunotherapy.^{53,54} Anti-IgE therapy, which neutralizes free IgE, has shown only modest and transient benefits in food allergy.⁵⁵ Thus,

development of improved and effective therapies for PNA is an ongoing challenge.

COMPLEMENTARY AND ALTERNATIVE MEDICINE IN THE UNITED STATES

A National Institutes of Health (NIH)/National Center for Complementary and Alternative Medicine (NCCAM) survey reported that >50% of Americans have used some form of complementary or alternative medicine (CAM) treatment. The chronic nature of allergic diseases and the lack of satisfactory treatment lead many families of children with allergy and asthma to seek CAM treatments.⁵⁶ Although the results of surveys on CAM use by children vary between the studies, the reported rates by children with asthma ranges from 33% to 89%.⁵⁶ A recent survey reported that a majority of pediatricians believe that their patients have a positive attitude toward, and are using, CAM. Because patients consider CAM therapies to be safe and effective, pediatricians would also consider referring patients to a CAM practitioner and receiving more education on CAM⁵⁷ so that they might better discuss the implications of using these therapies and potentially improve adherence to the prescribed medication regimen, and allergy and asthma management.⁵⁸ Increased use of CAM by allergy patients is also a driving force for allergists to gain more insight into CAM. The American Academy of Allergy, Asthma and Immunology (AAAAI) Complementary & Alternative Practice in Allergy Committee conducted a survey of allergists in 2007. Of 450 responders, 80% were interested in learning more about CAM. The participants clearly reflected their interest in learning more about various CAM modalities, with 88% extremely interested in herbal and botanical medicine. This highlights the need to develop safe and effective CAM therapy. The AAAAI Natural Medicines Comprehensive Database provides assistance for allergists in their practices (<http://www.aaaai.org/members.stm>). The AAAAI CAM committee promotes CAM research in allergy and asthma and provides insight into integrative medicine practice.

TRADITIONAL CHINESE HERBAL MEDICINES FOR ASTHMA AND FOOD ALLERGY

Traditional Chinese medicine (TCM) has a long history of human use in China and other Asian countries, such as Korea and Japan, for treating and preventing

disease, and is part of mainstream medicine in these countries. The main components of TCM include herbal therapy, acupuncture, acupressure/massage, mind-body therapy, and dietary therapy. Because TCM is part of Asian mainstream medicine, the cost is covered by insurance in these countries.

Traditional Chinese Medicine is also beginning to play a role in the healthcare system in the United States. Acupuncture needles have been approved by the US Food and Drug Administration (FDA) as a medical device. However, herbal medicines are viewed as dietary supplements and their cost is not covered by insurance. In recent years, the FDA has provided guidance for investigating botanical drug products, including complex formulas containing several herbs, focusing on efficacy, safety, and consistency.⁵⁹ The NIH/NCCAM define TCM as Whole Medical Systems⁶⁰ and provides grants to support clinical and basic research on CAM. Several publications, including ours, indicate that TCM has potential for treating asthma,⁶¹ managing allergic rhinitis,⁶² and improving quality of life of atopic dermatitis patients.⁶³ Therefore, in the near future, some TCM remedies may become “botanical drugs,” or prescription drugs via clinical investigation.

Since 2005, several controlled clinical studies of antiasthma herbal remedies, including antiasthma herbal medicine intervention (ASHMI),⁶⁴ Ding Chuan Tang,¹⁷ and the Chinese formula STA-1⁶⁵ have been published. Of these, ASHMI is the only antiasthma TCM herbal product that received FDA investigational new drug (IND) approval and entered clinical trials in the United States. Research into ASHMI's active compounds is actively being pursued. Research on TCM herbal therapy for food allergy is limited. The Food Allergy Herbal Formula-2 (FAHF-2) also received FDA IND approval and is undergoing clinical trials for multiple food allergies. Thus, this review is not a systematic review or meta-analysis, but rather a focus on up-to-date translational studies of safety, efficacy, and mechanisms of action of the herbal interventions ASHMI and FAHF-2 for asthma and food allergy, respectively, in murine models and clinical trials. In addition, potential effects of TCM on food allergy-associated recalcitrant eczema, based on case review, have also been included.

DEVELOPMENT OF ANTI-ASTHMA HERBAL MEDICINE INTERVENTION AS BOTANICAL DRUG FOR TREATMENT OF ASTHMA

The antiasthma herbal medicine intervention is an extract of 3 Chinese herbal medicines: Ling

Zhi (*Ganoderma lucidum* [*G. lucidum*]), Ku Shen (*Sophora flavescens* [*S. flavescens*]), and Gan Cao (*Glycyrrhiza uralensis* [*G. uralensis*]). It was derived from a 14-herb formula, MSSM-002, that has been used in China-Japan Friendship Hospital for treating childhood asthma. We tested ASHMI's pharmacological and immunological actions and mechanisms with in vivo and in vitro models and began to investigate its safety and efficacy in asthma patients (Table 1). Animal research was conducted in accordance with the NIH guidelines for the care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee. The human studies have been approved by the Mount Sinai School of Medicine Institutional Review Board. Each subject provided informed consent. The following are the major findings in preclinical and clinical studies related to ASHMI.

Preclinical Studies: Actions and Mechanisms of Action of Antiasthma Herbal Medicine Intervention in Animal Model

Broad Actions of Antiasthma Herbal Medicine Intervention in Animal Model

Zhang *et al.*²¹ investigated the pharmacological and immunological actions of ASHMI in a murine asthma model. In this study, BALB/c mice were sensitized weekly intraperitoneally twice with ovalbumin (OVA) and alum followed by 3 weekly intratracheal challenges with OVA, then 2 consecutive daily challenges (fourth and fifth challenges) 4 weeks later. To determine whether ASHMI affected the progression of chronic asthma, ASHMI was administered intragastrically (i.g.) twice daily for 6 weeks. ASHMI abolished the early-phase airway response, which was associated with significantly reduced plasma and bronchoalveolar lavage fluid (BALF) histamine and leukotriene C4 levels, and decreased plasma OVA-specific IgE levels. It was also shown that late-phase airway responses were prevented, which was evidenced by significantly reduced numbers of BALF eosinophils, decreased collagen content of lungs, fewer goblet cells in the airways, as well as reduced levels of the Th2 cytokines IL-4, IL-5, IL-13 but increased interferon (IFN)- γ , IL-10, and transforming growth factor β (TGF- β) levels in BALF. A potential direct effect of ASHMI on airway constriction was demonstrated by inhibition of acetylcholine (ACh)-induced constriction of murine tracheal rings *ex vivo*. This effect was not mediated by β 2-adrenergic receptor stimulation. ASHMI also increased production of the potent smooth muscle relaxer

Table 1. Major Known Actions and Mechanisms of ASHMI and FAHF-2.

ASHMI (IND)	FAHF-2 (IND)
Animal model (BALB/c mice) Reduces bronchoconstriction, airway inflammation and remodeling; BALF histamine & LTC₄, IL-4, IL-5, IL-13 levels, and serum IgE levels^{66,112} Increases BALF IFN-γ, IL-10, and TGF-β levels¹¹² Long-lasting post-treatment tolerance to antigen exposure¹¹³ High safety profile¹¹² Mechanistic studies Mouse cell lines and tracheal rings <i>G. uralensis</i> flavonoids inhibits Th2 cytokine production by memory Th2 cells (D10 G4.1, Th2 clone)⁸¹ <i>G. lucidum</i> triterpenoids inhibit TNF-α production by mouse macrophage (RAW267.4)¹¹⁴ <i>G. uralensis</i> flavonoids inhibit TNF-α production by brain microglial cells (BV2)¹¹⁵ <i>S. flavescens</i> flavonoids inhibit Ach-induced ASM contraction⁹⁰ Human cell lines and primary cells <i>G. uralensis</i> flavonoids inhibit eotaxin production by human fetal lung fibroblasts (HFL-1)⁷⁹ <i>S. flavescens</i> flavonoids inhibit IgE production by human myeloma cells (U266)¹¹⁶ ASHMI increases PGI₂ production by HASM¹¹² ASHMI inhibits IL-5, and increases IL-10 production by PBMCs from asthma patients⁹⁵ Clinical trials RCT, dose-escalation safety study. Adult 18 individuals (ASHMI, n = 12; placebo, n = 6); 1-week treatment duration⁹³ Major findings ASHMI is safe and well tolerated RCT, 91 individuals (ASHMI, n = 45; prednisone, n = 46); 4-week treatment duration⁶⁴ Major findings ASHMI significantly improved lung function (FEV₁ and PEF) but less effective than prednisone; reduced symptom scores and β2 agonist use; suppressed IgE and Th2 responses; no adverse effects on the major organs Unlike prednisone, ASHMI had no significant effect on body weight and no negative effects on adrenal function; a beneficial immunoregulatory effect on Th1/Th2 balance. RCT, 51 children aged 5–11 years (ICS + ASHMI, n = 25; ICS + placebo, n = 26 (ICS alone)); 3-month treatment duration⁹⁴ Major findings ICS+ASHMI was safe and well tolerated, and more effective than ICS alone in improving clinical symptoms	Animal model (C3H/HeJ mice) Completely prevents PN anaphylaxis and histamine release¹⁰⁰ Reverses established PNA¹⁰¹ Pharmacological and synergistic effect of constituents in FAHF-2⁹⁹ Prolonged post-treatment protection against PN anaphylaxis via CD8+ T cells¹⁰² High safety profile¹⁰⁰ Mechanistic studies Mouse and rat cell lines FAHF-2 exhibits long-lasting beneficial effects on T cells and B cells in vivo¹⁰² FAHF-2 suppresses IgE-mediated proliferation and FcϵRI expression, and histamine release by MC/9 mast cells¹⁰³ Phellodendri alkaloids inhibit IgE-mediated degranulation of mast cell/basophils cells (RBL-2H3) via blocking the Syk signaling pathway¹⁰³ Human cell lines and primary cells Phellodendri alkaloids inhibit IgE production by human B cells¹⁰³ FAHF-2 alkaloid-rich fraction suppresses human primary mast cell degranulation in response to IgE analog and complement stimulation¹⁰³ Clinical trials RCT, acute phase I. 18 individuals (FAHF-2, n = 12; placebo, n = 6), ages 12–45 years with PN and/or tree nut, fish, and shellfish allergy; 1-week treatment duration¹¹⁷ Major findings Safe and well tolerated Major findings Ex vivo immunological study showed FAHF-2 treatment reduced IL-5, no changes in IFN-γ secretion by PBMCs compared with placebo In vitro immunological study showed reduction of IL-5 and induction of IL-10 secretion by PBMCs after 3-day culture with FAHF-2 Open-label extended phase I, FAHF-2, n = 14, 6-month treatment duration¹¹⁸ Major findings Safe and well tolerated Inhibited ex vivo basophil activation after 4 and 6 months of treatment Trend of reduction of basophil and eosinophil numbers Phase II study (RCT) is ongoing

Abbreviations: Ach, acetylcholine; ASHMI, anti-asthma herbal medicine intervention; ASM, airway smooth muscle; β 2AR, β 2 adrenergic receptor; BALF, bronchoalveolar lavage fluid; FAHF-2, Food Allergy Herbal Formula-2; Fc ϵ RI, Fc ϵ receptor; FEV₁, forced expiratory volume of the first second; *G. lucidum*, *Ganoderma lucidum*; *G. uralensis*, *Glycyrrhiza uralensis*; HASM, human airway smooth muscle cells; ICS, inhaled corticosteroid (budesonide); IFN, interferon; IgE, immunoglobulin E; IL, interleukin; IND, investigational new drug; PBMCs, peripheral blood mononuclear cells; PEF, peak expiratory flow; PGI₂, prostaglandin I₂; PN, peanut; PNA, peanut allergy; RCT, randomized controlled trial; *S. flavescens*, *Sophora flavescens*; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor α .

prostacyclin or prostaglandin I₂ (PGI₂), indicating that PGI₂ plays a role in ASHMI-mediated airway relaxation.

Antiasthma Herbal Medicine Intervention Is Effective in Later-Onset Asthma

Busse *et al.*⁶⁶ subsequently investigated the effects of ASHMI on characteristics of allergic asthma in an aged mouse model of late-onset asthma. Although asthma is typically characterized as a childhood-onset disease, it also develops later in life. Airway disease may be more severe in later-onset asthma compared with earlier-onset disease⁶⁷; hospitalization and mortality rates are higher, and there is also a higher systemic steroid requirement in patients aged >65 years.^{68,69} Thus, later-onset asthmatic patients may be at increased risk for oral corticosteroid-induced potential adverse effects. Osteoporosis, increased fracture risk, muscle weakness, glucose intolerance, and easy bruising are of particular concerns in older patients. Therefore, developing nonsteroidal interventions for late onset asthmatic patients is important. In this study, ASHMI treatment of young and aged OVA mice resulted in significantly decreased lung inflammation, airway mucous cell metaplasia, and messenger RNA copy numbers of the mucin gene MUC5AC, as well as reduced collagen production.

Antiasthma Herbal Medicine Intervention Provides More Persistent Benefits Than Dexamethasone

Srivastava *et al.*^{23,24} evaluated the persistence of ASHMI's beneficial effects following discontinuation of therapy as and found that AHR and pulmonary eosinophilic inflammation did not recur following OVA re-challenge up to 8 weeks post-therapy. Decreased allergen-specific IgE and Th2 cytokine levels and increased IFN- γ levels also persisted at least 8 weeks post-therapy. In contrast, dexamethasone-treated mice exhibited similarly reduced airway inflammation, Th2 cytokines, and OVA-specific IgE levels immediately after therapy, but were not protected at 8 weeks post-therapy. IFN- γ levels in BALF, and serum corticosterone levels were reduced by dexamethasone treatment. To determine the contribution of IFN- γ to ASHMI effects, mice were given IFN- γ -neutralizing antibody concomitantly with ASHMI. The ASHMI effects were eliminated by neutralization of anti-IFN- γ antibody, but not anti-TGF- β antibody during therapy.

In studies involving animal models of airway hyper-reactivity, Srivastava et al. showed that antiasthma herbal medicine intervention provides more persistent benefits than dexamethasone.

Mechanistic Studies: Chemical and Biological Characterization of Active Compounds in Antiasthma Herbal Medicine Intervention

Research on herbal medicine development presents unique challenges because TCM formulas frequently contain many unknown components. The FDA has issued guidelines for industrial botanical products focusing on safety and efficacy.⁵⁹ Standardization of raw herbs, the manufacturing process, and the final product, as well as the safety data on limit testing of pesticides, heavy metals, and microbials, are key components in obtaining approval. Use of high-performance liquid chromatography (HPLC) is recommended by the FDA as a means of monitoring the consistency of raw herbs and the final product. We generated an HPLC fingerprint of ASHMI and a characterization of corresponding peaks and chemical markers (Figure 2). However, the active compounds responsible for ASHMI's therapeutic effects are largely unknown.

Isolation and identification of active constituents will improve herbal-product quality control and lead to better understanding of the mechanisms of action. Modern chromatographic techniques, such as HPLC, together with mass spectrometry and nuclear magnetic resonance (NMR) techniques, make feasible the isolation, purification, and identification of chemical constituents from complex mixtures.^{70–72} A phytochemical database of Chinese herbal constituents and bioactive plant components has recently been established.⁷³ Of these phytochemical classes, alkaloids, polyphenols, and terpenes are the most frequently identified biologically active compounds in TCMs.⁷⁴ However, because most previous phytochemical studies of herbal medicines focused mainly on isolation and identification of new compounds, and because limited amounts of material were isolated, few isolated compounds are commercially available. Therefore, studies on pharmacological activities and mechanisms of action relevant to asthma are limited. The critical step to identifying active compounds in a mixture is isolation of

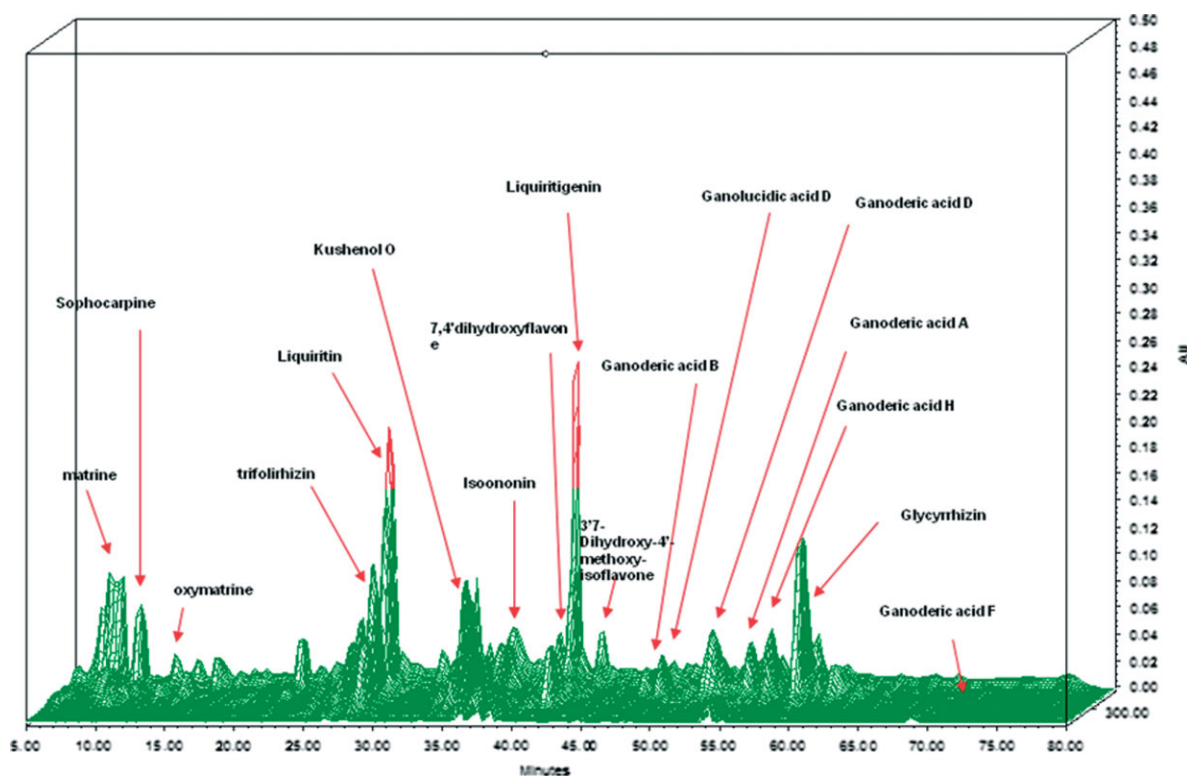


Fig 2. A 3-D HPLC-DAD fingerprint of the ASHMI extract.¹¹² Peak analysis and assignment were performed using standard samples and/or the LC-MS method. Matrine, sophocarpine, oxymatrine, kushenol O, and trifolirhizin correspond to the compounds in *Radix Sophorae Flavescentis*. Ganoderic acid B, D, A, H, and F and ganolucidic acid D correspond to *Ganoderma lucidum*. Liquiritin, liquiritigenin, 7,4'-dihydroxyflavone, isoononin, 3',7'-dihydroxy-4'-methoxy-isoflavone, and glycyrrhizic acid correspond to compounds in *Radix Glycyrrhizae*. **Abbreviations:** 3-D HPLC-DAD, 3-dimensional high-performance liquid chromatography with diode array detection; ASHMI, antiasthma herbal medicine intervention; LC-MS, liquid chromatography–mass spectrometry.

compounds. Given our understanding of pathological mechanisms of asthma, we have focused on isolation and identification of active compounds that contribute to ASHMI's anti-inflammatory effects and inhibit ASM contractility. The major findings are summarized below.

Glycyrrhiza uralensis Flavonoids Inhibit Eotaxin and Th2 Cytokine Production

Gan Cao (*G. uralensis*), commonly called licorice, is one of the most commonly used herbs in TCM. Gan Cao means “sweet herb.” Licorice products are most often consumed as flavoring and sweetening agents in food products. Based on phytochemistry, *G. uralensis* contains mainly terpenoids and flavonoids. The licorice triterpenoid glycyrrhizin and related compounds down-regulate production of inflammatory chemokines IL-8 and eotaxin 1 and stat 6 by a human lung fibroblast cell line.^{75,76} However, their effects on asthma have not been reported and other potential anti-inflammatory constituents such as flavonoids in *G. uralensis* have not been

fully investigated, partially due to lack of commercially available flavonoid compounds. Eosinophilic airway inflammation is a major feature of allergic asthma, and eotaxin is involved in recruitment of eosinophils to sites of antigen-induced inflammation in asthmatic airways.⁷⁷ Because in humans, lung fibroblasts are the major source of eotaxin,⁷⁸ inhibition of eosinophil recruitment by suppression of fibroblast eotaxin production is a potentially valuable approach to pharmacological intervention in asthma. Our group has conducted a systematic bioassay-guided purification of *G. uralensis*, which yielded 5 flavonoids: liquiritin, liquiritigenin, isoliquiritigenin, 7,4'-dihydroxyflavone, and isoononin. The structures of the compounds were established by ¹H and ¹³C NMR, and liquid chromatography–mass spectrometry (LC-MS) studies.⁷⁹ The potential ability of these isolated pure compounds, and of glycyrrhizin, to inhibit eotaxin secretion by human fetal lung fibroblasts was tested. We found that *G. uralensis* flavonoids inhibited eotaxin secretion and were more potent than the triterpenoid glycyrrhizin (commercially available)

and that liquiritigenin, isoliquiritigenin, and 7,4'-dihydroxyflavone were more effective than liquiritin. A dose-response study showed that the half maximal inhibitory concentrations (IC_{50}) of liquiritigenin, isoliquiritigenin, and 7,4'-dihydroxyflavone were 4.2, 0.92, and 0.21 $\mu\text{g/mL}$, respectively.⁷⁹

Consequently, we investigated the effects of these 3 flavonoids on a Th2 cell line (D10 G4.1) that produces robust Th2 cytokines to antigen (conalbumin) stimulation, and the in vivo effect of 7,4'-dihydroxyflavone. We found that 7,4'-dihydroxyflavone was also the most potent licorice flavonoid in suppression of Th2-cell cytokine production by D10 cells, which was associated with suppression of GATA-3 expression. 7,4'-dihydroxyflavone also showed inhibition of eosinophils and Th2 cytokines without suppression of $IFN-\gamma$ in an animal model of asthma. Abstracts describing these results have been published.^{80,81}

Glycyrrhiza uralensis Flavonoids Inhibit Microglial Cell Tumor Necrosis Factor- α Secretion and Increase Brain-Derived Neurotrophic Factor Secretion

Anxiety and depression are often associated with poor asthma control and poor quality of life.^{82–87} The prevalence of serious psychological distress among adults with asthma (7.5%) is more than double that in the overall US population (3.0%) and in adults without asthma (2.6%).⁸⁸ Significant symptoms of anxiety are experienced by 20%–40% of adolescents with asthma.⁸⁹ Inflammatory cytokines such as tumor necrosis factor- α (TNF- α) in the peripheral and central nervous system are associated with anxiety. Some conventional medications used to treat asthma can enhance anxiety and depression.³⁶ ASHMI has been shown to decrease inflammation in asthma patients and a murine asthma model, and suppress TNF- α production by macrophages in vitro. Brain-derived neurotrophic factor (BDNF) has been suggested to be beneficial for anxiety/depression. We determined the effects of ASHMI and its purified compounds on production of TNF- α and BDNF by mouse microglial cells. In this study, BV-2 mouse microglial cells (1×10^5) were cultured in Dulbecco's modified Eagle medium 10%/fetal bovine serum media for 48 hours until confluent. The cells were then pretreated with various doses (15.6–500 $\mu\text{g/mL}$) of ASHMI or extracts of 3 individual herbs within ASHMI for 8 hours and stimulated with lipopolysaccharide (500 ng/mL) for 24 hours. In addition, individual compounds isolated from Gan Cao (GC-1–5) were also tested (0.78–25 $\mu\text{g/mL}$). Levels of TNF- α in the supernatants were measured by enzyme-linked immunosorbent assay

and viability was determined by the MTT assay. ASHMI produced dose-dependent inhibition of TNF- α secretion (IC_{50} : 116.8 $\mu\text{g/mL}$). Of the 3 herbs, Gan Cao produced significant inhibition of TNF- α at all doses tested (IC_{50} : 16.9 $\mu\text{g/mL}$). Ling Zhi at 500 $\mu\text{g/mL}$ and Ku Shen at 250 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$ showed significant inhibition of TNF- α levels. Of the 5 flavonoids isolated from Gan Cao, GC-2, GC-3, and GC-4 showed dose-dependent inhibition of TNF- α , and IC_{50} values were 1.18 $\mu\text{g/mL}$, 2.21 $\mu\text{g/mL}$, and 4.86 $\mu\text{g/mL}$, respectively, with GC-3 being the more effective. GC-3 also inhibited nuclear factor kappa β (NF- κB) and induction of BDNF. ASHMI and its licorice flavonoids have an anti-inflammatory effect on mouse microglia cells and enhance BDNF production. ASHMI may provide additional benefits for patients with asthma and comorbid anxiety/depression. Clinical studies are warranted.

Effects of Active Compounds from Antiasthma Herbal Medicine Intervention on Airway Smooth Muscle Contraction

Because we previously showed that ASHMI has a direct effect on ASM contractility, to determine the active constituents responsible for this action, we tested the effects of 3 individual constituents in ASHMI on constriction of murine tracheal rings isolated from OVA-sensitized and challenged mice (asthmatic mice). Only Ku Shen (*S. flavescens* Ait) exhibited potent inhibition of ASM contraction to Ach provocation. A dichloromethane-extracted fraction of this herb, containing mainly flavonoids, retained the inhibitory effect of Ku Shen on ASM contraction. We therefore focused on this fraction and generated 19 subfractions using preparative HPLC, 3 of which contain major peaks named 10S, 11S, and 12S. 11S significantly suppressed tracheal ring contraction induced by 10^{-6} M to 10^{-4} M Ach compared with that in physiological salt solution (PSS; sham)-treated tracheal rings ($P < 0.01$ – 0.05). 12S only showed a minor effect, and 10S showed no inhibitory effect on tracheal ring contraction at any tested dose of Ach. This study indicated that 11S might be the major player in ASHMI responsible for inhibition of Ach-induced ASM hypercontractility and might be a novel ASM relaxation agent for potential asthma treatment.⁹⁰ Further characterization of this compound by NMR is underway.

Clinical Studies

Wen *et al.*⁶⁴ reported the first double-blind, randomized, placebo-controlled trial investigating the

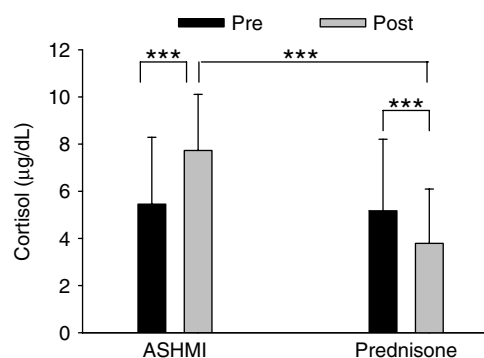


Fig 3. Effect of treatment with ASHMI or prednisone on serum cortisol levels determined by radioimmunoassay. To ensure consistency of the results, all blood samples were drawn between 7:30 and 8:30 A.M. (before treatment and 48 hours after treatment). Data are expressed as mean $\pm$ SD. *** indicates P value <0.001 . Normal level is 8–23 $\mu\text{g/dL}$. **Abbreviations:** ASHMI, antiasthma herbal medicine intervention; SD, standard deviation.

efficacy and tolerability of ASHMI in 91 patients aged 18–60 years with moderate to severe symptomatic asthma as compared with oral prednisone therapy. Subjects in the ASHMI group ($n = 45$) received oral ASHMI capsules (4 capsules, t.i.d., 0.3 g/capsule) and placebo tablets similar in appearance to prednisone. Subjects in the prednisone group ($n = 46$) received oral prednisone tablets (20 mg q.d. in the morning) and ASHMI placebo capsules. Treatment was administered daily over 4 weeks. This study found that post-treatment lung function (values for forced expiratory volume in 1 second FEV1 and peak expiratory flow PEF) was significantly improved in both the ASHMI ($64.9 \pm 6.3.6$ to $84.2 \pm 6.5.0$; $P < 0.001$) and prednisone ($65.2 \pm 6.3.7$ to $88.4 \pm 6.8.0$; $P < 0.001$) groups. The improvement was slightly but significantly greater in the prednisone group ($P < 0.05$). There was a significant and a similar degree of reduction in clinical symptom scores in both treated groups (median [range], ASHMI: 5.0 [4–8] to 2.0 [0–4], $P < 0.001$; prednisone: 5.0 [4–7] to 2.0 [0–4], $P < 0.001$), use of β_2 -bronchodilators (median [range], ASHMI: 4.7 [3.5–5.7] to 0.9 [0.14–2.3], $P < 0.001$; prednisone: 4.7 [3.5–5.6] to 0.6 [0.3–1.0], $P < 0.001$). ASHMI had no significant effect on body weight (increases in body weight post-therapy: 2.8 kg in the prednisone group vs 0.8 kg in ASHMI). Further, serum IgE levels and Th2 cytokine levels were significantly reduced in both groups. However, in contrast with prednisone, ASHMI significantly increased serum IFN- γ levels.

An additional interesting finding in the study by Wen *et al.* was a beneficial effect of ASHMI treatment on adrenal function. Pretreatment cortisol levels were slightly below normal (6–23 $\mu\text{g/dL}$) in both groups. After treatment, subjects in the prednisone treatment

group showed a significant reduction in serum cortisol levels (5.1 ± 3.0 to 3.7 ± 2.3 $\mu\text{g/dL}$, $P < 0.001$). In contrast, patients in the ASHMI treatment group showed increased levels of serum cortisol (5.4 ± 2.8 to 7.7 ± 2.3 $\mu\text{g/dL}$, $P < 0.001$) (Figure 3), which were within the normal range. The difference between groups was statistically significant ($P < 0.001$). Thus, ASHMI restored adrenal function. This result might be attributed to glycyrrhizin (a component of Gan Cao), which affects the conversion of cortisol to cortisone by inhibition of 11- β -hydroxysteroid dehydrogenase enzyme activity.⁹¹ Gan Cao has been used for adrenal insufficiency.⁹² However, it is also possible that the increase in cortisol levels may be due to the lack of suppression of adrenal function. Further research is required to understand the precise mechanisms. No significant adverse effects were observed. All hematological, electrocardiographic, and liver and kidney function test results were normal in both groups. Thus, ASHMI appeared to be effective and well tolerated, and may offer benefits comparable with standard corticosteroid therapy for some patients. Kelly-Piper⁹³ *et al.* conducted a double-blind, placebo-controlled, dose-escalation safety trial (phase I) in 18 adult asthma individuals and showed

In a study comparing antiasthma herbal medicine intervention (ASHMI) with prednisone in the treatment of moderate to severe symptomatic asthma, in which the subjects showed pretreatment serum cortisol levels slightly below normal, prednisone significantly reduced cortisol levels further whereas ASHMI increased them to the normal range. ASHMI thus restored adrenal function.

that ASHMI is safe and well tolerated. All subjects' laboratory test results (complete blood counts and chemistry) were within the normal range. Busse *et al.*⁹⁴ reported a study that determined the safety, tolerability, and immunological benefits of complementary ASHMI in children ages 5–14 years with persistent asthma with or without allergic rhinitis. Subjects were randomly assigned to receive standard ICS treatment (budesonide) plus ASHMI as complementary therapy (standard + ASHMI group, $n = 28$) or ICS treatment plus placebo (standard + placebo, $n = 28$). Fifty-one asthma patients completed the trial,

26 in the complementary ASHMI group and 25 from the standard group. We found that patients in the complementary ASHMI group showed significantly greater reduction of serum total IgE ($P < 0.05$) and higher serum levels of IFN- γ ($P < 0.001$) and cortisol levels ($P < 0.05$) after 3 months of treatment as compared with the standard + placebo group.

Patil *et al.*⁹⁵ showed that ASHMI in vitro treatment of peripheral blood mononuclear cells (PBMCs) from patients with moderate to severe asthma ($n = 31$) for 72 hours significantly suppressed IL-5 production without affecting IFN- γ and cell viability, and increased IL-10 production to antigen stimulation compared with untreated PBMCs. This in vitro study provided direct evidence that ASHMI has immunomodulatory effects. A phase II study involving 60 patients is underway.

Taken together, these studies showed that ASHMI was safe and well tolerated in human trials and showed beneficial clinical and immunological effects. However, the study size is generally small. ASHMI also showed multiple beneficial effects on asthma-related pathological mechanisms in animal model of asthma. Identification of active compounds in ASHMI will enhance our understanding of the pharmacological mechanisms of ASHMI and accelerate the process for developing ASHMI as botanical drug in the United States. Asthma is a serious disease, the lack of control of which can be very dangerous; until very-high-level double-blind randomized controlled trials are conducted on ASHMI, clinicians should adhere to current pharmaceutical guidelines, possibly with the incorporation of herbal therapeutics.

DEVELOPMENT OF FOOD ALLERGY HERBAL FORMULA-2 AS A BOTANICAL DRUG FOR TREATMENT OF FOOD ALLERGY

The term “food allergy” is not described in the classical TCM literature, but a food allergy-like phenomenon has long been recognized. Wu Mei Wan is a classical Chinese formula for treating intestinal parasite infection, first documented by Dr. Zhang Zhong Jing in a classical Chinese medical text compiled approximately 2000 years ago. This formula, along with other classical TCM formulas, have been translated into English by Bensky *et al.* in *Chinese Herbal Medicine: Formulas and Strategies*.⁹⁶ The indications of Wu Mei Wan include intermittent attacks of abdominal pain, a stifling sensation, irritability, and warmth in the chest (anxiety), accompanied by

vomiting, diarrhea after eating, and cold hands and feet. Based on our understanding of food allergy clinical expression and immunopathological mechanisms, and TCM formula principles and updated research in TCM and allergy, we developed Food Allergy Herbal Formula-1 (FAHF-1) derived from Wu Mei Wan, containing 11 herbs. We then refined and improved that formula into FAHF-2, which contains 9 herbs. The pharmacological and immunological actions and mechanisms of FAHF-2 were tested in both in vivo and in vitro models, and phase I studies were conducted in patients with PN and/or tree nut, fish, shellfish, and sesame allergies (Table 1). Animal research was conducted in accordance with the NIH guidelines for the care and use of laboratory animals and approved by Institutional Animal Care and Use Committee. The human studies have been approved by the Mount Sinai School of Medicine Institutional Review Board. Each subject provided informed consent. The following are the major findings in preclinical and clinical studies related to FAHF-2.

Preclinical Studies

Effect of Food Allergy Herbal Formula-2 on Peanut Allergy in Animal Model

Peanut allergy is potentially life-threatening. We previously found that FAHF-1 blocked PN-induced anaphylaxis in a murine model when challenged immediately post-therapy.⁹⁷ We then tested whether FAHF-2 was equally effective, and if so, whether protection persists after therapy is discontinued. The raw herb quality was ascertained according to the standards required by the Pharmacopoeia of the People's Republic of China.⁹⁸ Based on organoleptic and microscopic examination, the raw herbs used in FAHF-2 were identified as *Prunus mume*, *Zanthoxylum schinifolium*, *Angelica sinensis*, *Zingiber officinalis*, *Cinnamomum cassiae*, *Phellodendron chinense*, *Coptis chinensis*, *Panax ginseng*, and *Ganoderma lucidum*. The quality and ratio of herbs used has been described previously.⁹⁹ Product quality was monitored by HPLC fingerprinting according to the FDA's Guidance for Industry Botanical Drug Products.⁵⁹

In this study, mice allergic to PN treated with FAHF-2 for 7 weeks were challenged 1, 3, or 5 weeks post-therapy. The results showed that 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, decreased core body

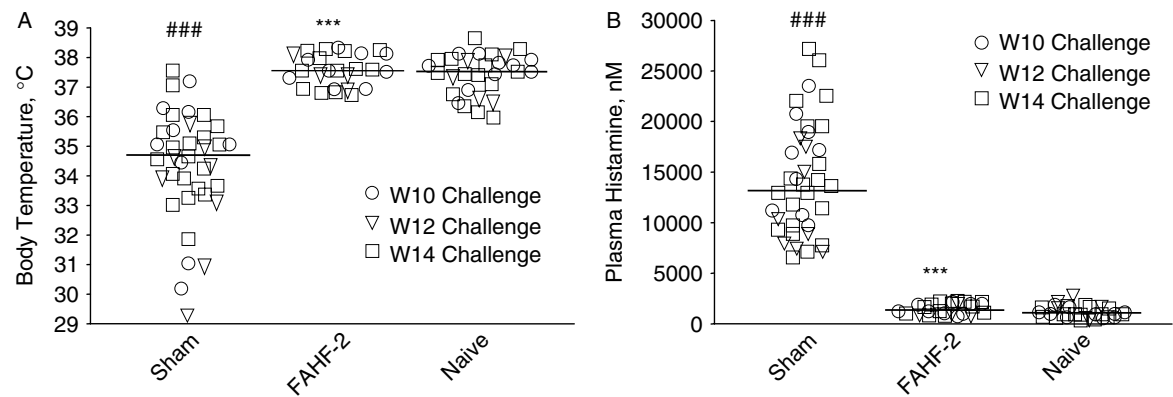


Fig 4. (A) FAHF-2 prevented a drop in core body temperatures. Rectal temperatures were measured 25 minutes following i.g. PN challenge in mice challenged at weeks 10 (○), 12 (▽), and 14 (□). Bars indicate the medians of temperature from totals of 5 sets of experiments (Sham, n = 38; FAHF-2, n = 26; Naïve, n = 29). ### indicates $P < 0.001$ versus Naïve, and *** indicates $P < 0.001$ versus Sham. (B) FAHF-2 blocked histamine release. Data are plasma histamine levels 30 minutes post-challenge, the same mice as in (A). ### indicates $P < 0.001$ versus Naïve, and *** indicates $P < 0.001$ versus Sham. **Abbreviations:** FAHF-2, Food Allergy Herbal Formula-2; i.g., intragastric; PN, peanut.

temperatures, or elevation of plasma histamine levels was observed in FAHF-2–treated mice in 5 separate experiments Figure 4. The IgE levels were significantly reduced by FAHF-2 treatment, and remained significantly lower as long as 5 weeks post-therapy. Splenocytes from FAHF-2–treated mice showed significantly reduced IL-4, IL-5, and IL-13, and enhanced IFN- γ production to recall PN stimulation in vitro. FAHF-2 also showed a high safety margin.¹⁰⁰ We also found FAHF-2 induced PN tolerance in mice after establishment of peanut allergy. This effect was associated with up-regulation of IFN- γ by CD8+ T cells.¹⁰¹

Food Allergy Herbal Formula-2 (FAHF-2) prevents and reverses peanut allergy in an animal model of peanut anaphylaxis.

Pharmacologic and Immunologic Effects of Individual Herbs in Food Allergy Herbal Formula-2 on Peanut Allergy

We then investigated the pharmacological actions of individual herbs comprising FAHF-2 on PN-induced anaphylactic reactions in a murine model of PNA, and determined if all 9 herbs were necessary to prevent anaphylactic reactions, or if a simplified formula containing fewer herbs would be equally effective. Our results showed that some individual herbs reduced PN-induced anaphylactic symptoms, but no

single herb offered full protection from anaphylactic symptoms equivalent to FAHF-2 (Table 2). The herbs had highly variable effects on histamine release, as well as PN-specific serum IgE and IgG2a levels. The herbs also had variable effects on IL-4, IL-5, and

Table 2. Effect of FAHF-2 and Its Individual Constituents on PN-Induced Anaphylaxis.

Herbal Treatment	Anaphylactic reactions n/total	% of mice with a reaction	Anaphylactic score Median (range), p values vs Sham
Sham	9/11	81	2 (0–4)
FAHF-2	0/8	0	0 (0), p < 0.001
HB	1/4	25	0 (0–2), p = 0.008
GJ	3/5	60	2 (0–2), p = 0.058
HL	3/5	60	2 (0–4), p = 0.058
CJ	5/8	63	2 (0–4), p = 0.140
LZ	3/4	75	2.5 (0–3), p = 0.590
WM	6/7	86	2 (0–4), p = 0.387
GZ	6/7	86	2 (0–3), p = 0.330
DG	5/5	100	3 (2–3), p = 0.640
RS	4/4	100	3 (2–3), p = 0.386
Naïve	0/15	0	0 (0–0), p < 0.001

Abbreviations: CJ, Chuan Jiao; DG, Dang Gui; FAHF-2, Food Allergy Herbal Formula-2; GJ, Gan Jang; GZ, GuiZhi; HB, Huang Bai; HL, Huang Liang; LZ, Ling Zhi; PN, peanut; RS, Ren She; Sham, PN allergic mice fed water; WM, Wu Mei. Anaphylactic reaction rates and median scores obtained 30 minutes after oral PN challenge using this scoring system: 0, no signs; 1, scratching and rubbing around the snout and head (mild reaction); 2, puffiness around the eyes and snout, diarrhea, pilarrecti, reduced activity, and/or decreased activity with increased respiratory rate (moderate reaction); 3, wheezing, labored respiration, cyanosis around the mouth and tail (severe reaction); 4, no activity after prodding, or tremor and convulsion (near-fatal reaction); 5, death.

IFN- γ levels. A simplified formula comprising only the most efficacious individual herbs showed only partial efficacy and was not able to reproduce the effects of FAHF-2. This finding suggested that component herbs of FAHF-2 may be working synergistically to produce the curative therapeutic effects demonstrated by the whole formula, which appears to be the best option for future clinical trials.⁹⁹

Food Allergy Herbal Formula-2 Silences Peanut-Induced Anaphylaxis for a Prolonged Post-Treatment Period via IFN- γ -Producing CD8+ Cell

Because, in mice, FAHF-2 treatment reestablished tolerance to PN after PN hypersensitivity was fully established, and because this effect persisted for 4 weeks post-therapy,¹⁰¹ we sought to determine if FAHF-2-mediated protection could be extended long-term and explored the mechanisms underlying its persistent immunomodulatory effects. In this study, PN-allergic mice received FAHF-2 daily orally for 7 weeks, and then received 7 oral PN challenges at 4- to 10-week intervals over 36 weeks. For mechanistic studies, some mice received CD4+ or CD8+ T cell-depleting antibodies or IFN- γ -neutralizing antibodies. Anaphylactic symptoms, body temperatures, and plasma histamine levels were recorded following each challenge, and PN-specific Ig levels and cytokine profiles of splenocytes, mesenteric lymph node cells, and purified CD4+ and CD8+ T cells were determined.

The results showed that FAHF-2 treatment protected mice from anaphylaxis for >36 weeks after discontinuing treatment (Table 3). PN-specific IgE levels were reduced up to 50%, whereas IgG2a levels were increased up to 60%, and these effects persisted over time. Clinical protection was also

accompanied by marked suppression of plasma histamine levels, which was associated with significant suppression of mast cell degranulation (Figure 5). At week 50, the experiment was terminated and mice were sacrificed. Splenocytes and mesenteric lymph node cells from these mice showed significant suppression of Th2 cytokines (IL-4, IL-5, and IL-13) and enhancement of IFN- γ production (Figure 6). Furthermore, Th2 cytokine production by CD4+ T cells from FAHF-2 treated mice was reduced up to 75%, whereas CD8+ T cell IFN- γ production was increased by up to 85% at the final challenge. Neutralization of INF- γ and depletion of CD8+ T cells markedly attenuated FAHF-2 efficacy.¹⁰² This study showed that FAHF-2 provides long-term protection from anaphylaxis by inducing a beneficial shift in allergen-specific immune responses mediated largely by elevated CD8+ T cell IFN- γ production.

FAHF-2 silences peanut-induced anaphylaxis for a prolonged post-treatment period via interferon- γ producing CD8+ T cells.

Food Allergy Herbal Formula-2 Protection Against Peanut Anaphylactic Reactions Is via Inhibition of Mast Cells and Basophils

Mast cells and basophils are key effector cells of IgE-mediated anaphylactic reactions. Using a well-established murine model of PN anaphylaxis, we found that FAHF-2 abrogated anaphylactic reactions in mice, and this protection persisted for ≥ 36 weeks after therapy was discontinued.¹⁰² The

Table 3. Persistent Protection of FAHF-2 Against PN-Induced Anaphylaxis.

Challenge (Time-point)	PNA/Sham		PNA/FAHF-2		Naive	
	Individual Score (N = 12–13)	Median Score	Individual Score (N = 8)	Median Score	Individual Score (N = 5–10)	Scores Median
1 st (W14)	13/13 (2–4)	3	0/8 (0)	0*	0/5 (0)	0
2 nd (W18)	13/13 (3–4)	4	0/8 (0)	0*	0/5 (0)	0
3 rd (W22)	13/13 (3–4)	4	0/8 (0)	0*	NC	ND
4 th (W28)	13/13 (2–4)	4	0/8 (0)	0*	NC	ND
5 th (W34)	13/13 (3–4)	4	0/8 (0)	0*	NC	ND
6 th (W30)	13/13 (3–5)	4	0/8 (0)	0*	NC	ND
7 th (W50)	12/12 (3–4)	4	3/8 (0–2)	0*	0/10 (0)	0

Abbreviations: FAHF-2, Food Allergy Herbal Formula-2; PN, peanut; PNA, peanut allergy; Sham, PN-allergic mice fed water.

$P < 0.05$ versus Sham.

Anaphylactic signs were evaluated 30 minutes following oral challenge utilizing the scoring system previously described in Table 1.

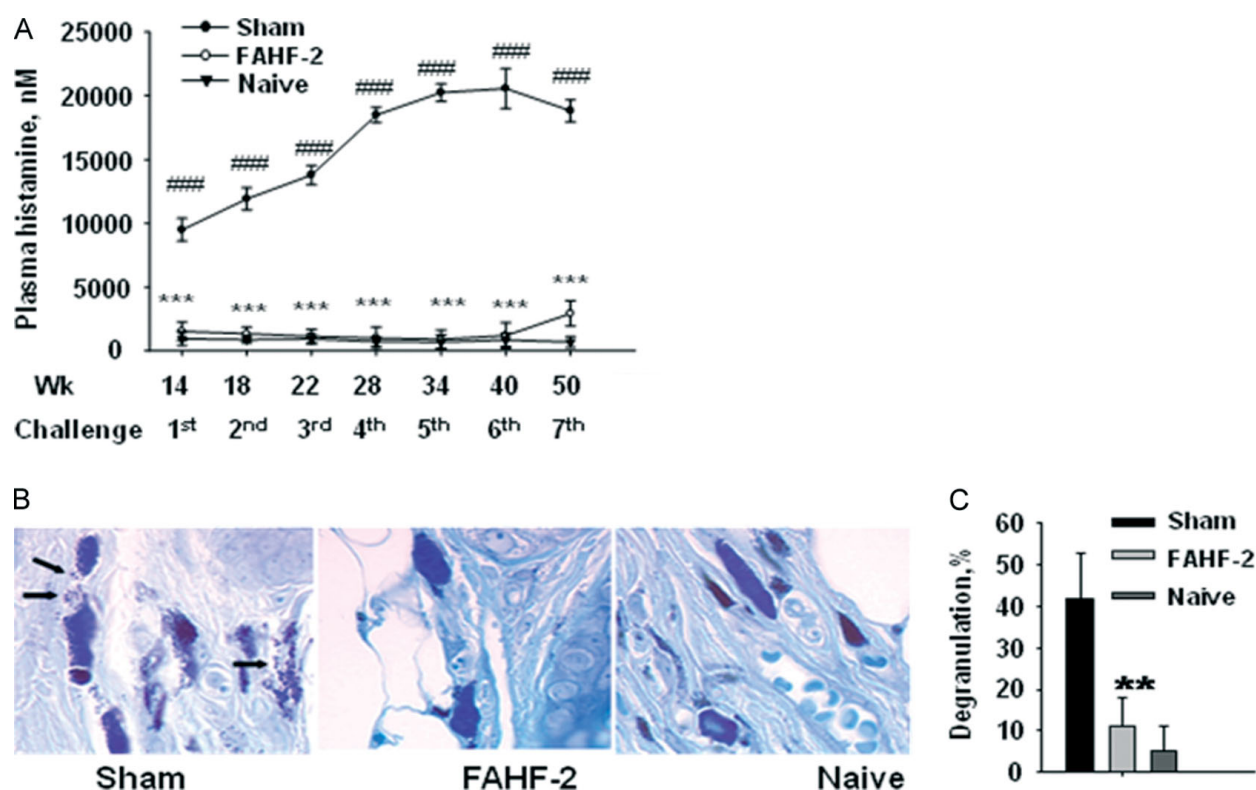


Fig 5. FAHF-2 treatment prevented histamine release and mast cell degranulation. (A) Plasma histamine levels. Blood was collected immediately after scores and temperatures were recorded following each post-therapy challenge, and plasma was obtained. Histamine levels were measured using enzyme immunoassay. Data points indicate group means $\pm$ SEM, n = same as indicated above. The scale of the x axis is not linear. (B). Illustration of mast cell degranulation, Ear tissue samples taken 40 minutes after seventh challenge and stained with toluidine blue. Mast cells were considered degranulated if ≥ 5 granules appeared outside cell body. Arrows indicate deregulated mast cells. The image was captured using $100\times$ lens. (C) Percent of degranulated mast cells. Mast cells were counted using light microscopy at $100\times$ lens. A total of 500 cells were counted from 3 sections of each ear sample for calculation of the percentage of degranulated cells. Data points indicate group means $\pm$ SEM, n = 4–5 mice/group from 1 set of experiment. ** indicates $P < 0.01$, *** indicates $P < 0.001$ versus Sham, and ### indicates $P < 0.001$ versus Naive. **Abbreviations:** FAHF-2, Food Allergy Herbal Formula-2; SEM, standard error of the mean.

increased IFN- γ production by CD8+ T cells, as shown previously, may be an important mechanism underlying FAHF-2–modulated potent and long-term protection.¹⁰² However, neutralization of IFN- γ or depletion of CD8+ T cells blocked the suppression of IgE and Th2 cytokine production induced by FAHF-2, whereas protection from anaphylaxis still existed up to 4 weeks post-therapy. We therefore hypothesized that FAHF-2 may also directly inhibit mast cells/basophils. To confirm this possibility, we investigated whether FAHF-2 inhibits mast cell/basophil numbers and IgE-mediated activation. In this study, PNA mice were treated with FAHF-2 i.g. for 7 weeks and challenged (i.g.) with PN 1 day and 4 weeks post-treatment. Peripheral blood basophil numbers and peritoneal mast cell numbers and Fc ϵ RI expression were determined. Direct effects of FAHF-2 on murine mast cell line MC/9, and effects of 4 fractions and 3 compounds

isolated from FAHF-2 on rat basophilic leukemia cells (RBL-2H3) and human skin mast cells degranulation, and on the IgE-mediated Syk signaling pathway, were determined. The results showed that although all sham-treated PNA mice developed anaphylaxis, FAHF-2–treated PNA mice were protected against anaphylaxis following PN challenge at 1 day and 4 weeks post-therapy. Reduction of peripheral blood basophil numbers began after 1 week of treatment and continued for ≥ 4 weeks post-therapy. The number of Fc ϵ RI expression by peritoneal mast cells was also significantly decreased 4 weeks post-therapy. FAHF-2–treated MC/9 cells showed significantly reduced IgE-induced Fc ϵ RI expression, Fc ϵ RI γ mRNA subunit expression, proliferation, and histamine release upon challenge. Fraction 2 (F2) from FAHF-2 inhibited RBL-2H3 cell and human mast cell degranulation. Three compounds from F2, berberine, palmatine, and jatrorrhizine, inhibited

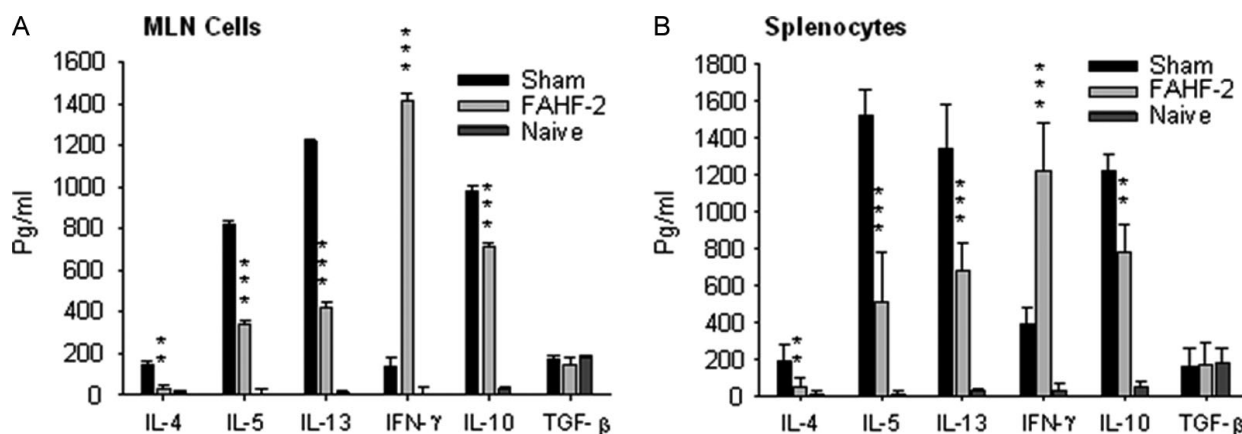


Fig 6. FAHF-2 treatment reduced Th2 cytokine levels and increased IFN- γ secretion by specific modulation of CD4+ and CD8+ T cells. Immediately following evaluation of clinical effect and blood drawing following the seventh challenge (week 50), MLN cells and SPCs were collected from each group of mice. Single MLN cells and SPCs were prepared and stimulated with CPE for 72 hours. Cytokines in MLN culture supernatants (A) and SPC culture supernatants (B) were measured by ELISA. Data are shown as means $\pm$ SEM of pooled cultures from representative of 1 of 2 experiments measured in triplicate (n = 5 mice per group). ** indicates $P < 0.01$, *** indicates $P < 0.001$ versus Sham. **Abbreviations:** CPE, crude peanut extract; ELISA, enzyme-linked immunosorbent assay; FAHF-2, Food Allergy Herbal Formula-2; IFN- γ , interferon- γ ; MLN, mesenteric lymph node; SEM, standard error of the mean; SPCs, splenocytes; Th2, T helper cell type 2.

FAHF-2 protection against peanut anaphylactic reaction is via inhibition of mast cells and basophils.

RBL-2H3 cell degranulation via suppressing Syk phosphorylation.¹⁰³ This study demonstrates that FAHF-2 reduction of basophils and mast cell numbers, as well as suppression of IgE-mediated mast cell activation, may contribute to FAHF-2's persistent protection against PN anaphylaxis.

Clinical Studies

Safety and Immunological Effects in an Acute Phase I Study

Because FAHF-2 has been shown to have potent therapeutic effects in an animal model of PN anaphylaxis and a high safety profile in preclinical study, and because all individual herbs in FAHF-2 have a long history of human use and are lawfully used in the United States, FAHF-2 received approval of IND status by the FDA to study its safety and efficacy in patients ages 12–45 years with PN and/or tree nut, fish, and shellfish allergies. This clinical study included both phase I (safety study) and phase II (efficacy study). Phase I study included acute phase I study and extended phase I study. We have completed both phase I studies. The acute phase I is a randomized, double-blinded, placebo-controlled, dose-escalation

trial to evaluate the safety and tolerability of FAHF-2 in subjects with food allergy. In this study, subjects received 1 of 3 doses of FAHF-2 or placebo: 2.2 grams (4 tablets), 3.3 grams (6 tablets), or 6.6 grams (12 tablets) 3 $\times$ /day for 7 days. Four active and 2 placebo subjects were treated at each dose level. Vital signs, physical examination, laboratory data, pulmonary function tests, and electrocardiogram data were monitored. Immunomodulatory studies were also performed. Nineteen food-allergic participants were included in the study. Two subjects (1 FAHF-2 and 1 placebo) reported mild GI symptoms. One patient withdrew from the study due to an allergic reaction that was unlikely related to the study medication. No significant differences were found in vital signs, physical examination, laboratory data, pulmonary function tests, and electrocardiogram data obtained at pretreatment and post-treatment visits. Significantly decreased IL-5 levels were found in the active treatment group after 7 days. In vitro studies of PBMCs cultured with FAHF-2 also demonstrated a significant decrease in IL-5 and an increase in IFN- γ and IL-10 levels. Wang J, Patil S, Yang N, Ko J, Lee J, Noone S *et al.* Safety, tolerability, and immunologic effects of a food allergy herbal formula (FAHF-2) in food allergic individuals: a randomized, double-blinded, placebo-controlled, dose escalation phase I study. *Ann Allergy Asthma Immunol* 2010; 105(1): 75–84. This data showed that FAHF-2 is safe and well tolerated by subjects with food allergy and also showed beneficial immunoregulatory effects, which is favorable for induction of food allergy tolerance.

Clinical Safety of Food Allergy Herbal Formula-2 and Its Inhibitory Effect on Basophils From Patients With Food Allergy: Extended Phase I Study

Basophils comprise <1% of leukocytes but are critical to allergic reactions, as are tissue mast cells. CD63 expression, a marker of basophil activation,¹⁰⁴ correlates with release of histamine from intracytoplasmic granules and is triggered by IgE and allergen cross-linking of FcεRI receptors. The basophil activation test is a flow cytometry–based assay that detects basophil CD63 expression, requires the use of very small quantities of blood, and does not require isolation of cells. The real-time flow cytometry readout makes this assay a convenient tool for longitudinal clinical studies.^{51,105–107} A recent study by Jones *et al.* found that basophil activation was significantly reduced by 4–6 months of OIT⁵¹ and that inhibition of basophils, but not IgE, correlated with clinical protection. Because PN, tree nut, fish, and shellfish allergies are typically lifelong, chronic treatment is essential. To further ensure the safety and tolerability of this formulation prior to phase II study, we conducted an open-label, single-dose, 6-month extension of the phase I study. Although the primary outcome of this study was to evaluate the long-term safety and tolerability of FAHF-2, based on substantial animal study results, and the fact that this is a 6-month study, we determined the effects of FAHF-2 on basophils in individuals before, during, and after FAHF-2 treatment. Of the 18 patients enrolled, 14 completed the study. No significant drug-associated differences in laboratory parameters, pulmonary function studies, or electrocardiographic findings before and after treatment were found. There was a significant reduction ($P < 0.05$) in basophil CD63 expression in response to ex vivo stimulation after 6 months of FAHF-2 treatment. There was also a trend toward a reduction of circulation basophil and eosinophil numbers after treatment. It was concluded that FAHF-2 was safe, well tolerated, and had an inhibitory effect on basophils in an extended phase I clinical study. A controlled phase II study is warranted.

In summary, in an animal model of PNA, FAHF-2 treatment completely protected PNA mice from anaphylaxis despite multiple oral PN challenges for >6 months—one-quarter of the mouse lifespan. This effect was associated with sustained suppression of Th2 responses (IgE and Th2 cytokines) and enhancement of Th1 responses (IgG2a and IFN- γ). Elevation of IFN- γ via CD8+ T cells and persistent inhibition of basophils may contribute to FAHF-2's long-term protection. Clinical study showed that FAHF-2

is safe and well tolerated by patients with food allergy.

Treatment with FAHF-2 completely protected peanut-allergic mice from anaphylaxis despite multiple oral peanut challenges for >6 months—one-quarter of the mouse lifespan.

There was significant suppression of Th2 responses, basophil activation, and increased IFN- γ and IL-10, suggesting beneficial immunological responses, which are favorable for food allergy treatment. Therefore, FAHF-2 appeared to have multiple targets on food allergy–related immunopathological mechanisms, as illustrated in Figure 1.

TRADITIONAL CHINESE MEDICINE FOR FOOD ALLERGY–ASSOCIATED RECALCITRANT ECZEMA

Atopic dermatitis (AD) is a chronic, inflammatory, and pruritic skin disease that affects up to 20% of children.^{108,109} Regardless of dietary modifications, daily hydration therapy, and topical corticosteroids, some of these children continue to suffer from severe skin lesions in the form of recalcitrant eczema. Clinically, 40% of refractory, moderate-to-severe AD is associated with food hypersensitivity.¹¹⁰ Traditional Chinese medicine has been used for centuries to treat eczema; however, an effect on recalcitrant eczema has not been previously reported. Wisniewski *et al.*¹¹¹ reported a case series of 14 patients (median age, 5.4 years [interquartile range: 0.5–52]) with persistent recalcitrant AD who were treated with TCM at Ming Qi Natural Health Center in Manhattan between August 2006 and May 2008. This was a retrospective analysis of patients with AD who received TCM consisting of Shizheng Herbal Tea, Herbal Bath Additive, Herbal Cream, and acupuncture. The Scoring Atopic Dermatitis (SCORAD) Index for AD severity was calculated; scores <15 are considered mild, 15–40 moderate, and >40 severe.⁹⁹ The Dermatology Life Quality Index (DLQI) was calculated on a scale of 0–30, with a score of 30 representing the highest impairment to quality of life.

The baseline-median (IQR) SCORAD and DLQI scores were 89/42–103 and 17/10–30 compared with 110–62 and 10–14 after a median of 8 months 3–24 treatment (Student *t* test; $P < 0.0001$ and $P <$

0.0001). A 60%–90% reduction in SCORAD was reached in 13 of 14 patients after 3.3 months (1.6–8.6). A >50% improvement in DLQI scores was achieved in 13 of 14 patients in 2.4 months (0.7–5.9) (Figure 5). Peripheral eosinophilia decreased from 1000 ± 700 mc/L to 500 ± 200 ($P = 0.03$) with no change in total blood counts. No abnormality of liver and kidney function was observed. Patients reported a reduction in use of steroids, antibiotics, and antihistamines within 3 months of TCM treatment. These results showed that TCM is both a safe and effective treatment for patients with persistent AD, especially those with severe disease and significant life quality impairment.¹¹¹ Food allergy–associated eczema is often accompanied by elevation of total and food allergy–specific IgE levels. Interestingly, there was a reduction of food-specific IgE levels following eczema clinical improvement (Li *et al.*; manuscript in preparation). It is possible that improvement of food allergy–associated eczema might be beneficial for food allergy treatment. At present, there is no other report about the effect of TCM effect on food allergy–associated eczema beyond this retrospective clinical analysis. Although the clinical effects are promising, controlled study is required to further investigate the efficacy and safety of these herbal treatments for food allergy–associated recalcitrant eczema.

CONCLUSION

Traditional Chinese medicine is beginning to play a role in the US healthcare system. There is increasing scientific evidence demonstrating that TCM has potential for treating asthma and food allergy, and associated conditions. Our goals are to accelerate clinical trials and to identify the active compounds that target specific asthma and food allergy pathways, and comorbid conditions such as asthma–associated anxiety and food allergy–associated severe and recalcitrant eczema.

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DISCLOSURES

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