

The Chinese herbal medicine formula MSSM-002 suppresses allergic airway hyperreactivity and modulates T_H1/T_H2 responses in a murine model of allergic asthma

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Background: Asthma is a major public health problem worldwide, and the morbidity and mortality of asthma have increased in the past two decades. The reputed efficacy, low cost, and relative absence of side effects of traditional Chinese medicines (TCMs) have led to increasing interest in the use of TCMs for the treatment of asthma in Western countries. However, there are few well-controlled scientific studies on the efficacy, safety, and mechanisms of action of TCMs used to treat asthma.

Objective: The goal of this study was to investigate the effects of the Chinese herbal medicine formula MSSM-002, derived from TCMs used to treat allergic asthma, on a well-characterized mouse model of allergic asthma.

Methods: Mice sensitized intraperitoneally and challenged intratracheally with conalbumin were treated with MSSM-002 24 hours after the first intratracheal challenge. Dexamethasone-treated, saline solution sham-treated, and naive mice served as controls. The effects of MSSM-002 on allergic airway hyperreactivity, inflammation, antigen-specific antibody production, lung histologic features, and cytokine profiles were evaluated.

Results: MSSM-002 treatment virtually eliminated airway hyperreactivity and markedly reduced the total number of cells and the percent eosinophils in bronchoalveolar lavage fluids compared with the sham-treated group. Lung histologic features showed that MSSM-002 reduced inflammation and mucus production. These effects were equivalent to the effects of dexamethasone, but in contrast to the overall immunosuppressive effects of dexamethasone MSSM-002 treatment decreased antigen-specific IgE, IL-4, IL-5, and IL-13 levels without suppressing IgG2a and IFN- γ synthesis.

Conclusion: MSSM-002 exhibits anti-airway hyperresponsiveness, anti-airway inflammation, and immunoregulatory effects

on T_H1/T_H2 responses, which may be useful for treatment of allergic asthma. (*J Allergy Clin Immunol* 2000;106:660-8.)

Key words: Traditional Chinese medicine, murine model of allergic asthma, T_H1/T_H2

Asthma is a major public health problem worldwide, and asthma morbidity and mortality have increased over the last two decades, particularly in Western countries.^{1,2} Although the reasons for this increased incidence are unknown, there is a clear association between atopy and asthma.³ Antigen-induced IgE production, airway inflammation, and airway hyperresponsiveness or airway hyperreactivity (AHR)⁴⁻⁶ have been well documented in patients with allergic asthma and in animal models,^{7,8} and increasing evidence suggests that the T_H2 -type cytokines IL-4, IL-5, and IL-13, produced by activated CD4⁺ T cells, play a central role in the pathogenesis of allergic asthma.^{8,9}

With the increased understanding of the pathogenesis of asthma, anti-inflammatory therapies have become important therapeutic strategies for asthma treatment. Corticosteroids are the most potent nonspecific anti-inflammatory agents, and they are known to produce substantial improvement in objective lung functions of asthmatic subjects. Because of the many undesirable side effects of systemic corticosteroids, inhaled corticosteroids are used as the first line of treatment for asthma and are an effective means of reducing inflammation and bronchial constriction in the majority of patients.¹⁰ Although the most frequently reported side effects of inhaled corticosteroids are local effects, systemic side effects also have been reported. Adrenal suppression, decreased bone metabolism, and decreased growth are of particular concern in children, in whom asthma is increasing in frequency.^{11,12} Thus there is a need for new or alternative approaches to the control of this disease.

Complementary or alternative medicine (CAM) approaches such as traditional Chinese medicine (TCM), used in China, Japan, and other Asian countries for centuries, are attracting increasing interest in Western countries as possible additional treatments of a variety of diseases, including asthma. A recent survey of 1584 South Carolina adults found that 44% had used CAM including herbal medicines, during the past year (1999) and that

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US Provisional Patent Application (reference No. 202834-0027) regarding MSSM-002 has been filed.

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Abbreviations used

AHR:	Airway hyperresponsiveness or airway hyperreactivity
APTI:	Airway pressure–time index
BALF:	Bronchoalveolar lavage fluid(s)
CA:	Conalbumin
CAM:	Complementary or alternative medicine
Con A:	Concanavalin A
Dex:	Dexamethasone
DNP:	Dinitrophenyl
RT:	Room temperature
TCM:	Traditional Chinese medicine

more than 60% of these perceived CAM therapy to be very effective.¹³ A review of the use of TCM in the treatment of asthma and allergies by But and Chang¹⁴ and an extensive summary of publications in English relating to herbal and herbal-derived medications used in asthma and allergy recently published by Bielory and Lupoli¹⁵ reported that some antiasthma TCM formulas reportedly improved symptoms and medical scores, increased peak expiratory flow values, or reduced the need for steroids and β_2 -agonists in patients, and reduced AHR and eosinophilia in animal models of asthma. However, the role of herbal medicines in asthma therapy in Western medicine is still uncertain. More research is needed to investigate the effectiveness and the mechanisms of action of herbal medicines.

We previously generated a mouse model of allergic asthma that exhibits pulmonary eosinophilia, AHR, and increased antigen-specific IgE associated with increased IL-4 and IL-5 levels in bronchial lavage fluids (BALF). We used this model in the current study to evaluate possible effects of the herbal formula MSSM-002 (original name MTSD) on eosinophilic inflammation and antigen-induced AHR and to explore its possible effects on immune responses. We found that MSSM-002 suppressed AHR and reduced airway inflammation. These effects were equivalent to dexamethasone (Dex) treatment. Furthermore, MSSM-002, unlike Dex, which suppressed both T_H2 and T_H1 responses, primarily suppressed the T_H2 -type response.

MATERIAL AND METHODS

Mice and reagents

Male AKR/J mice (6 weeks old) purchased from the Jackson Laboratory (Bar Harbor, Me) were maintained in the animal facility at Mt Sinai School of Medicine. Standard guidelines for laboratory animal care were followed.¹⁶ Conalbumin (CA), concanavalin A (Con A), Dex, and dinitrophenyl (DNP)-conjugated albumin were purchased from Sigma (St Louis, Mo). Antibodies for ELISAs were purchased from the Binding Site and PharMingen (San Diego, Calif). Anti-DNP IgE, IgG2a, and IgG1 were purchased from Accurate Scientific (Westbury, NY).

MSSM-002 preparation

MSSM-002, a formula developed in our laboratory, is based on an empiric prescription, *Ja Wai San Zi Tang*, used in the pediatric department of the China-Japan Friendship Hospital in Beijing to

treat asthma and bronchitis in children with coughing, wheezing, and chest tightness. MSSM-002, an extract of 14 herbs, was prepared according to classic TCM herbology methods as described in *Chinese Herbal Medicine: Formulas and Strategies*.¹⁷ Fig 1 shows some crude herb components of MSSM-002. A mixture of the first 13 components, Zi Su Zi (*Perilla frutescens*) 9 g, Ting Li Zi (*Descurainia sophia*) 9 g, Xing Ren (*Prunus armeniaca*) 9 g, Huang Qin (*Scutellaria baicalensis*) 9 g, Ku Sheng (*Sophora flavescens*) 9 g, Dang Gui (*Angelica sinensis*) 9 g, Bai Shao (*Paeonia lactiflora*) 9 g, Ge Gen (*Pueraria lobata*) 9 g, Jei Gen (*Platycodon grandiflorum*) 9 g, Gan Cao (*Glycyrrhiza uralensis*) 6 g, Da Zao (*Zizyphus jujuba*) 6 g, Sheng Jiang (*Zingiber officinale*) 6 g, and Zhen Zhu Mu (*Pteris margaritifera*) 9 g, was soaked in 660 mL of spring water for 30 minutes at room temperature (RT) and then boiled for 1 hour, resulting in a volume of 300 mL. Ling Zhi (*Ganoderma lucidum*) 6 g was boiled separately and lyophilized, and 150 mg of this lyophilate was added to the first decoction to produce the daily dose of medicine for a 10-year-old child. Aliquots of the final preparation were stored at -20°C and used within 20 days. On the basis of a conversion table of equivalent effective dose ratios from human to animal based on body surface area,¹⁸ the dose in this study was 1 mL per mouse twice daily.

Antigen sensitization, challenge, and treatment

Mice were sensitized by two intraperitoneal injections of 200 μg of CA and 2 mg of alum in 0.3 mL of PBS (1 week apart) as shown in Fig 2. Seven days after the last sensitization, mice were anesthetized and challenged intratracheally with 100 μg of CA in 0.05 mL of PBS followed by two more intratracheal challenges on days 20 and 30. Intratracheal challenge was performed as previously described.¹⁹ MSSM-002 (1 mL/mouse) was administered intragastrically 24 hours after the first antigen challenge and then twice daily for 17 consecutive days (Fig 2). Intragastric feeding was performed by means of a 25-gauge stainless steel blunt feeding needle (Fine Science Tool, Foster, Calif). Dex-treated mice received 0.5 mg/kg intraperitoneally daily, as described previously.²⁰ Sham-treated mice received saline solution intragastrically daily. Naive mice served as additional controls.

Measurement of late-phase airway responses

Three days after the last intratracheal antigen challenge, airway responsiveness was determined by measuring airway pressure changes after intravenous acetylcholine injection, as previously described.^{21,22} Mice were anesthetized with sodium pentobarbital (80 mg/kg) and ventilated with a tracheal cannula (18 gauge) at the rate of 120 breaths per minute and a constant tidal volume of air (0.2 mL) with an RSP1002 pressure-controlled respirator system (Kent Scientific, Litchfield, Conn). Muscle paralysis was induced by intravenous injection of decamethonium bromide (25 mg/kg). Airway pressure was measured with a pressure transducer through a port in the trachea. Two minutes after a stable airway pressure recording was established, acetylcholine was injected intravenously (50 $\mu\text{g/kg}$). The airway pressure changes were viewed and recorded for 4 minutes with VENTP software respiratory data acquisition system (Kent Scientific). The time-integrated changes in peak airway pressure referred to as the airway pressure–time index (APTI) (cmH_2O per second) were calculated and served as the measurement of airway responsiveness.

BALF preparation and cell differential counts

After airway response measurements were made, mice were killed and the lungs lavaged with 1.0 mL of ice-cold HBSS (without magnesium or calcium) and the BALF collected into chilled tubes

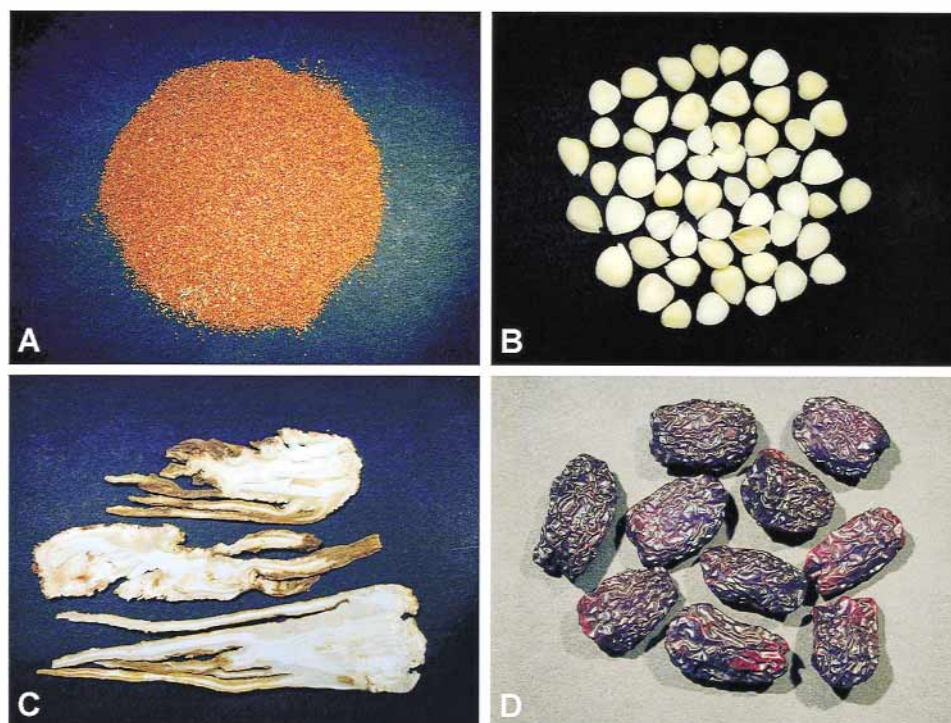


FIG 1. Illustration of some of the crude herb components of MSSM-002. **A**, Ting Li Zi (*D sophia*). **B**, Xing Ren (*P armeniaca*). **C**, Jei Jen (*P grandiflorum*). **D**, Da Zao (*Z jujuba*).

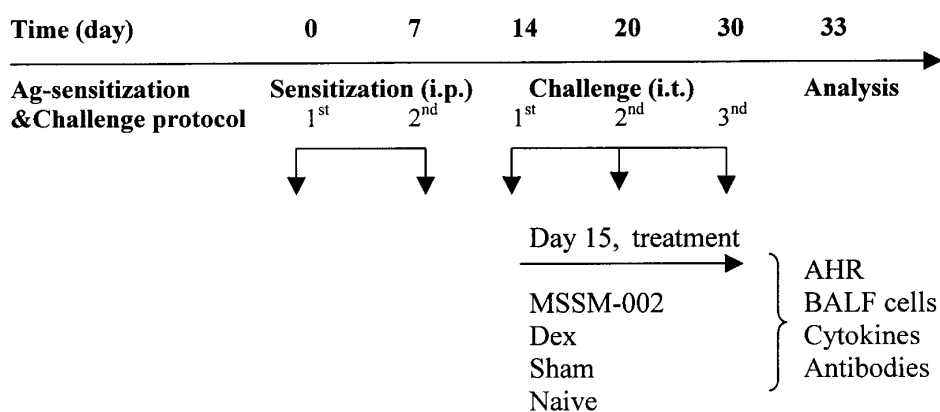


FIG 2. Experimental protocol. Mice were sensitized intraperitoneally (*i.p.*) with CA (200 µg/mouse) twice at weekly intervals and then challenged intratracheally (*i.t.*) on days 14, 20, and 30. Twenty-four hours after the first challenge, mice were treated with MSSM-002 (1 mL/mouse intragastrically twice daily), Dex (0.5 mg/kg intraperitoneally once daily), or saline solution (1 mL/mouse intragastrically twice daily) for 17 days. Naive mice served as additional controls. *Ag*, Antigen.

and centrifuged at 1000g for 10 minutes at 4°C. The total number of BALF cells was counted with use of a hemocytometer. Differential BALF cell counts were determined by microscopic evaluation of 500 cells per Diff-Quik (Dade Diagnostics of PR, Aguada, PR)-stained cytospin slide.

Histologic examination

Mice in each group (n = 4/group) were necropsied and the major internal organs fixed in neutral buffered formaldehyde. Five-micrometer paraffin sections were stained with hematoxylin and eosin and periodic acid-Schiff's reagent and examined microscopically.

Measurement of serum CA-specific antibodies

Sera were obtained from each group of mice immediately after APTI measurements and stored at -80°C. CA-specific IgE levels were measured by ELISA as described previously.²³

For measurement of CA-specific IgG2a or IgG1, plates were coated with CA and then blocked and washed as above. Samples (1:50 dilution) were added to the plates and incubated overnight at 4°C. Plates were washed, and biotinylated rat anti-mouse IgG2a or IgG1 mAbs were added and the plates incubated for an additional 45 minutes at RT.

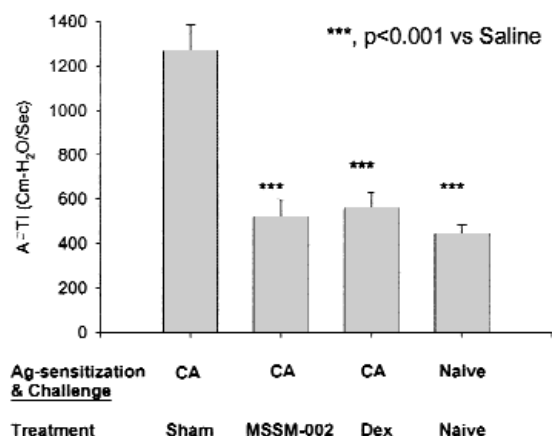


FIG 3. Effect of MSSM-002 on antigen (Ag)-induced AHR. Three days after the last antigen challenge, airway reactivity of mice in each group (saline solution, $n = 18$; MSSM-002, $n = 12$; Dex, $n = 13$; naive, $n = 17$) was determined by measuring the airway pressure change after acetylcholine challenge. The time-integrated changes in peak airway pressure referred to as the APTI (in cm H₂O-second) were calculated. Data are mean $\pm$ SEM for each group from three sets of experiments using four to six mice per group in each experiment.

After washing, avidin-peroxidase (Sigma, 1:1000 dilution) was added for an additional 15 minutes at RT. After washing, the reactions were developed with 2,2'-azine-bis(3-ethylbenzthiazoline-6-sulfonic acid) (KPL, Gaithersburg, Md) for 30 minutes at RT and read at 405 nm.

Because there is no commercially available mouse anti-CA antibody, the equivalent concentrations of antigen-specific IgE, IgG2a, and IgG1 were calculated by comparison with a reference curve generated with mouse mAbs, anti-DNP IgE, IgG2a, and IgG1 as described previously.²³ Briefly, DNP-albumin was coated at the same concentration as CA, and after overnight incubation at 4°C the plates were washed and blocked as described above. Ten serial 1:2 dilutions of murine anti-DNP IgE, IgG2a, or IgG1 antibodies were added, beginning with a concentration of 1000 ng/mL. Thereafter all steps were performed as described above. All analyses were performed in duplicate, and coefficients of variation >10% were repeated to ensure a high degree of precision.

Cell culture and quantification of cytokines

Immediately after APTI measurement, pooled spleen cells from four to five mice were isolated and suspended in complete culture medium (RPMI 1640 containing 10% FBS, 1% penicillin-streptomycin, and 1% glutamine). Cells (4×10^6 /mL/well) were cultured in 24-well plates in the presence of CA (50 μ g/mL). Cells cultured in the presence of Con A (2 μ g/mL) or medium alone served as positive and negative controls, respectively. Supernatants were collected after a 72-hour culture.

Levels of IL-4, IL-5, IL-13, and IFN- γ in spleen culture supernatants were determined by ELISA according to the manufacturer's instructions (PharMingen, San Diego, Calif), as previously described.²³

Statistical analysis

Data were analyzed with SigmaStat 2.03 statistical software (SPSS, Chicago, Ill). For all outcomes (except for BALF total cell numbers) the data of APTI, percent of BALF eosinophils, IgE, and IgG2a passed the test for normality. Differences between the treatment groups (sham, MSSM-002, and Dex) were determined by

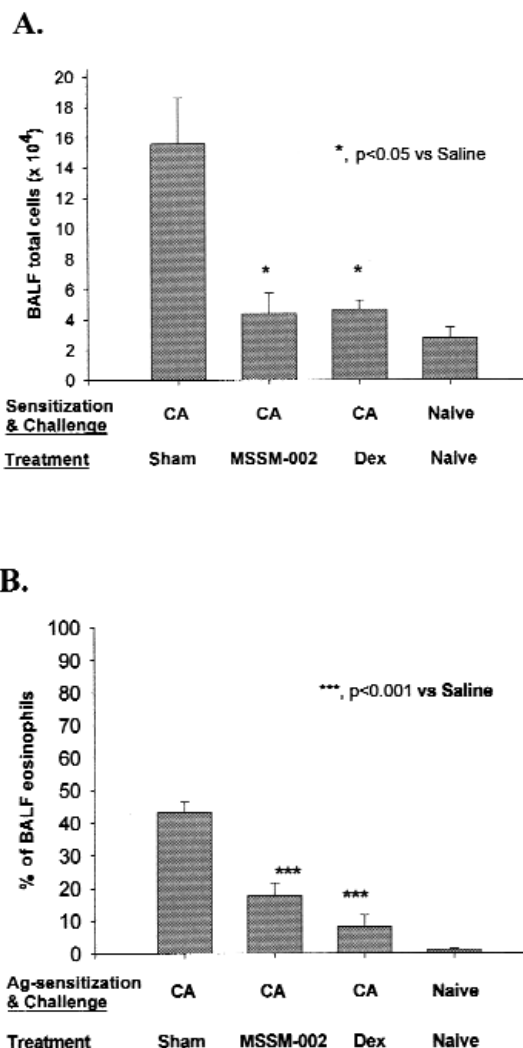


FIG 4. Effect of MSSM-002 on pulmonary inflammation. After airway response measurements, mice (saline solution, $n = 18$; MSSM-002, $n = 11$; Dex, $n = 12$; naive, $n = 16$) were killed and the lungs lavaged. **A**, Total cell numbers in BALF. **B**, Percent eosinophils in BALF. Data are mean $\pm$ SEM for each group from three sets of experiments using four to six mice per group in each experiment. Ag, Antigen.

ANOVA, and Bonferroni's t test for conservative correction was performed when ANOVA tests were significant at $P < .05$. For BALF total cell numbers, differences among the treatment groups (sham, MSSM-002, and Dex) were determined by use of the Kruskal-Wallis one-way ANOVA on ranks, followed by Dunn's method for all pairwise multiple comparison. A P value $< .05$ was considered statistically significant. The minimal number of mice in each group required to detect statistically significant differences in APTI levels is eight to nine by power analysis (power, 0.8 with $\alpha = .05$).

RESULTS

Effect of MSSM-002 on antigen-induced AHR

Three days after the last antigen challenge AHR was determined by measuring the airway pressure changes

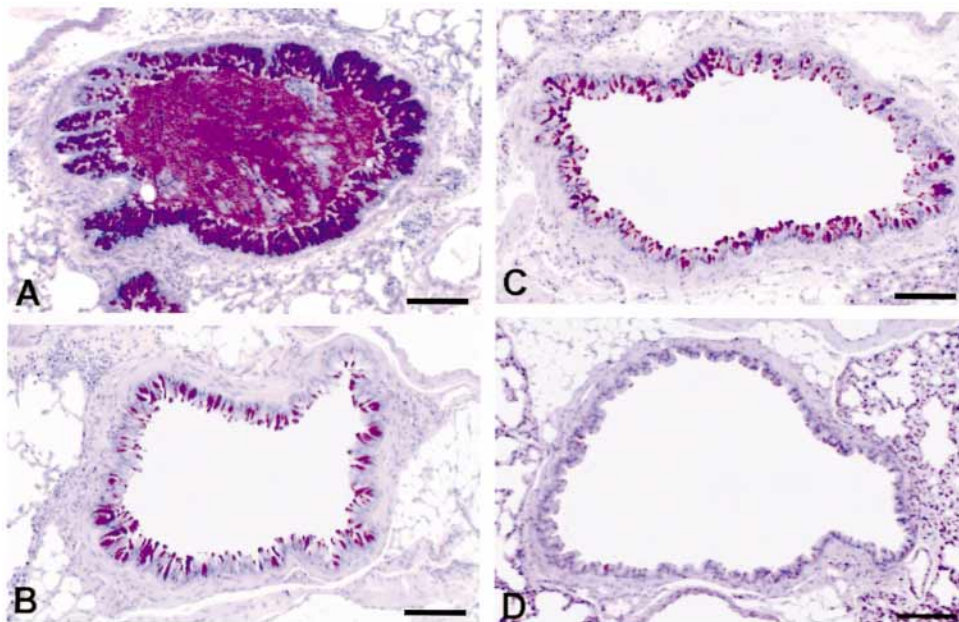


FIG 5. Lung histology. **A**, Goblet cell hyperplasia and mucus plug in airway from a saline solution sham-treated mouse. **B** and **C**, Reduced mucus production in airways of MSSM-002- and Dex-treated mice. **D**, Absence of goblet cells in airways of naive mice. Bars = 100 μ m.

over time after acetylcholine challenge, and the results are expressed as APTI. Consistent with our previous findings in this model,²² APTI levels of sham-treated, antigen-sensitized/challenged mice were markedly higher than those of naive mice, indicating the induction of AHR (Fig 3). APTI levels in MSSM-002- and Dex-treated groups were significantly lower compared with those of the sham-treated group ($P < .001$ vs sham, respectively) and were essentially the same as those in naive mice. There was no significant difference between APTI levels in the MSSM-002 and Dex treatment groups. These results demonstrated that, compared with sham treatment, MSSM-002, like Dex, virtually eliminated AHR in this model.

Effect of MSSM-002 on antigen-induced pulmonary inflammation

To determine whether suppression of AHR was associated with reduced inflammation, we measured the total number of cells and percent eosinophils in BALF. The total number of cells in the MSSM-002- and Dex-treated groups was significantly lower compared with the sham-treated group (Fig 4, A, $P < .05$ vs sham), and there was no statistically significant difference between MSSM-002 and Dex treatment. Although BALF from naive mice contained virtually no eosinophils, BALF from the sham-treated, antigen-sensitized, and challenged group contained 44% of eosinophils (Fig 4, B). Both MSSM-002 and Dex treatment significantly reduced the number of eosinophils in BALF ($P < .001$ vs sham). Although the anti-eosinophil effect of Dex appeared greater than that of MSSM-002, the difference in percent BALF eosinophils between these two treatment groups was not statistically significant.

Histologic examination

Mice from each group were necropsied, and sections of the major organs were examined by a pathologist unaware of their origin. No gross or histologic abnormalities were observed in tissues other than the lung. As previously described,²³ lungs from sham-treated/antigen-sensitized/challenged mice contained large numbers of peribronchial and perivascular eosinophils (data not shown), and numerous goblet cells and mucus plugs were present, particularly in the larger airways (Fig 5, A). In accord with BALF data, lungs from both MSSM-002- and Dex-treated animals contained markedly fewer inflammatory cells (data not shown) and no airway mucus plugging, although the number of goblet cells did not appear to be dramatically reduced by either treatment (Fig 5, B and C).

Effect of MSSM-002 on antigen-specific antibody responses

To determine the effects of MSSM-002 treatment on humoral immune responses, we measured serum CA-specific IgE and IgG2a. As shown in Fig 6, IgE levels were significantly, although not dramatically, decreased ($P < .01$). IgG2a levels were slightly increased in the MSSM-002-treated group compared with the sham-treated group but did not reach statistical significance. Dex treatment also significantly decreased IgE levels ($P < .01$ vs sham), but unlike MSSM-002 it also significantly reduced IgG2a levels ($P < .05$ vs sham). In addition, CA-specific IgG1 levels in both MSSM-002- and Dex-treated groups were also decreased compared with the sham-treated group (data not shown).

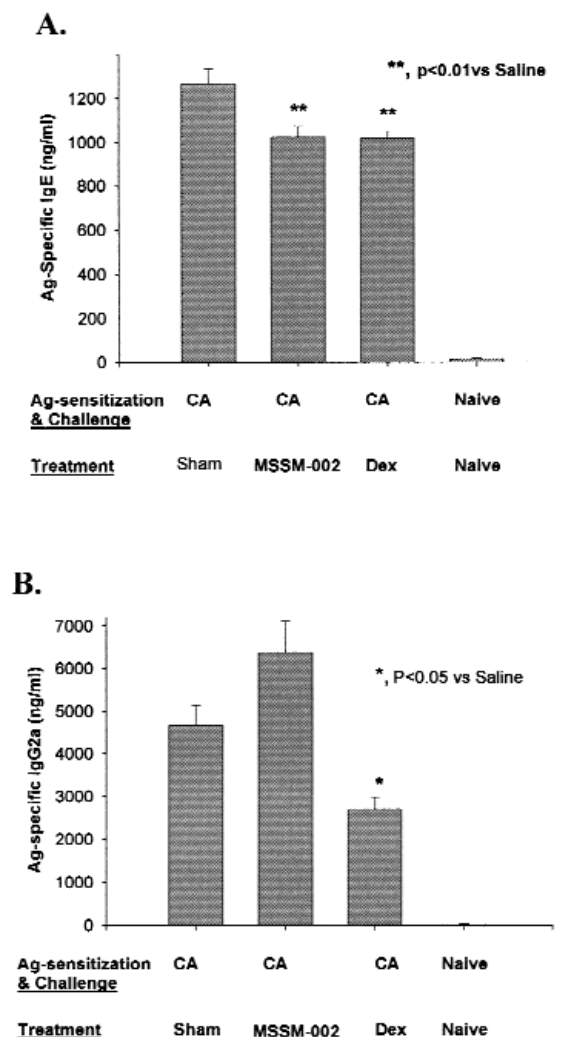


FIG 6. Serum CA-specific antibodies. Blood was obtained from each group of mice (saline solution, *n* = 16; MSSM-002, *n* = 11; Dex, *n* = 12; naive, *n* = 14) immediately after APTI measurements. Serum CA-specific antibody levels were determined by ELISA. **A**, IgE levels. **B**, IgG2a levels. Data are mean $\pm$ SEM for each group from three sets of experiments using four to six mice per group in each experiment. Ag, Antigen.

Effect of MSSM-002 on T-cell cytokine responses

To determine a possible effect of MSSM-002 on T-cell responses, we evaluated the effects of MSSM-002 and Dex on T-cell cytokine secretion by splenocyte cultures. As shown in Fig 7, IL-4, IL-5, and IL-13 levels were markedly increased, and IFN- γ levels were decreased in cultures from antigen-sensitized/challenged/sham-treated mice compared with normal controls, showing a predominant T_H2 response. However, IL-4 secretion was virtually abrogated, and IL-5 and IL-13 levels were reduced by approximately 40% in splenocyte cultures from MSSM-002-treated mice compared with cultures from sham-treated mice. Interestingly, CA-induced IFN- γ secretion by splenocytes from MSSM-002-treated

mice was increased by approximately 27% compared with cultures from sham-treated mice, and Con A-induced IFN- γ secretion was equivalent to that of splenocytes from naive mice. Splenocyte cultures from Dex-treated mice showed a different pattern. IL-4 secretion was virtually absent, IL-5 and IL-13 levels were markedly decreased, but CA-induced IFN- γ secretion was abrogated, and Con A-induced IFN- γ secretion was markedly lower than normal.

DISCUSSION

It has been reported that some antiasthma TCM formulas have therapeutic effects on allergic asthma.¹⁵ However, there are few well-controlled clinical studies and no previously reported animal studies in which TCM herbal remedies were administered after challenge of antigen-sensitized animals. In this study we have, for the first time, evaluated possible therapeutic effects of a TCM herbal formula on allergic airway responses in a well-characterized murine model of allergic asthma. We found that treatment with MSSM-002 beginning 24 hours after intratracheal antigen challenge of sensitized mice virtually eliminated AHR and that this effect was equivalent to that of Dex. These results suggest that MSSM-002 and similar TCM formulas may be useful alternative or complementary approaches for the treatment of allergic asthma.

MSSM-002 also exhibited anti-inflammatory properties as evidenced by reduction in the total numbers of cells and the percent of eosinophils in BALF. Lung histologic examination also showed that MSSM-002, like Dex, reduced inflammation and mucus hypersecretion, suggesting that the suppressive effect of MSSM-002 on AHR may, at least partially, be associated with reduction of airway inflammation and mucus production. However, despite total suppression of AHR, MSSM-002 did not eliminate inflammatory cells in the lung. Several previous studies also reported that a 40% to 50% reduction in BALF eosinophils was accompanied by markedly reduced AHR after a variety of interventions.^{24,25} It is likely that in animal models the number of activated eosinophils has to exceed a fairly high threshold to affect airway function. As in the case of eosinophils, goblet cells in the lung were not eliminated by either MSSM-002 or Dex. Previous studies using the antiallergy drug suplatast tosilate or IL-13 blockade also found nearly complete suppression of AHR but incomplete suppression of goblet cell hyperplasia.²⁵⁻²⁷ It is possible that the combination of partial suppression of eosinophilia and goblet cell hyperplasia might be sufficient to suppress AHR in this model. On the other hand, several studies have found no relationship between eosinophil levels or mucus hypersecretion and AHR.^{28,29} Thus other mechanisms such as MSSM-002 effects on smooth muscle or nerves may also be involved. Further study is required to delineate the mechanisms of action of this herbal formula on AHR.

IgE mediates the allergic response in humans as well as in animal models of allergic disease by triggering mast

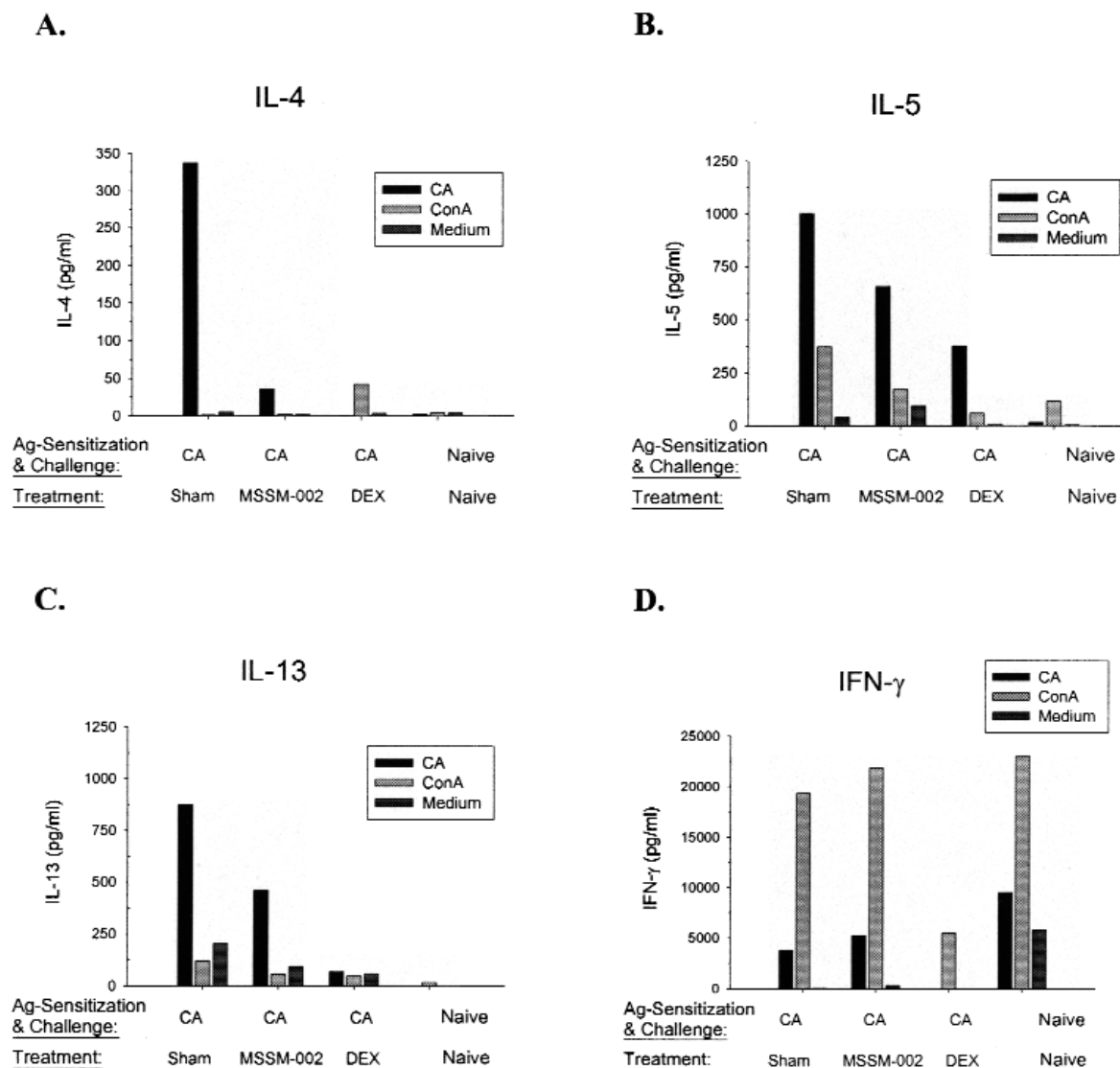


FIG 7. Cytokine production in spleen cell culture supernatants. Immediately after APTI measurement, pooled spleen cells from four to six mice in each group were isolated and cultured in complete culture medium in the presence of CA (50 μ g/mL), Con A (2 μ g/mL), or medium alone. Supernatants were collected after 72 hours of culture. Levels of IL-4, IL-5, IL-13, and IFN- γ were determined by ELISA. Results are mean values of duplicate assays. Ag, Antigen.

cell/basophil degranulation after relevant antigen exposure.³⁰⁻³³ IgG2a production, on the other hand, is considered to be beneficial.³⁴ Previous study showed that certain antiasthma TCM formulas, such as *Xiao Qing Long Tong*, inhibit passive cutaneous anaphylaxis in animal studies,³⁵ suggesting an immunotherapeutic effect on the allergic disease processes. We demonstrated that MSSM-002 treatment of sensitized and challenged mice significantly, although not markedly, reduced antigen-specific serum IgE. We also found that IgG1 levels were decreased and IgG2a levels were slightly increased by MSSM-002 treatment. Whether reduction of IgE (or IgG1) played any role in suppressing AHR in this model is unknown, but these findings provide experimental evi-

dence that this herbal formula exhibits an immunoregulatory effect that may be favorable for the treatment of allergic diseases.

As recently reviewed by Romagnani,⁹ T_H2 cytokines play a central role in the pathogenesis of asthma. IL-4/IL-13 promotes B-cell switching to IgE production and mucus hypersecretion. IL-5 has been shown to be the primary determinant of eosinophil priming, activation, recruitment, and survival. A previous study showed that the antiasthma herbal medicine *saiboku-to* inhibited antigen-induced eosinophilia and AHR as well as IgE (as indicated by inhibition of passive cutaneous anaphylactic reactions), and the authors hypothesized that the prophylactic effects of *saiboku-to* may be due to inhibition of

IL-5³⁶ or IL-4 synthesis.³⁷ However, no direct evidence supporting this possible mode of action was presented.

In this study we found that, compared with the sham-treated group, MSSM-002 down-regulated IL-4, IL-5, and IL-13 production by cultured splenocytes. These results demonstrate that MSSM-002 suppression of AHR and inflammation in this model is associated with specific down-regulation of T_H2 responses. Previous studies investigating the role of individual cytokines showed that anti-IL-4 or anti-IL-13 receptor antibodies suppressed antigen-induced AHR but not eosinophilic inflammation,^{38,39} whereas anti-IL-5 antibody administered after antigen challenge suppressed eosinophilic inflammation but had little effect on AHR.⁴⁰ More recently, Webb et al²⁹ showed that AHR is regulated by integrated IL-13, IL-4, and IL-5 signals. Because natural allergic airway reactions are likely mediated by a combination of T_H2 cytokines, MSSM-002 suppression of the three major T_H2 cytokines may offer some advantage over therapeutic administration of single antibodies against IL-4, IL-5, or IL-13 or their receptors.

As expected, Dex, one of the most potent corticosteroids, also suppressed antigen-induced AHR and eosinophilic inflammation in this model. However unlike MSSM-002, Dex suppressed T_H1 responses (IFN- γ /IgG2a) as well as T_H2 (IL-4, IL-5, and IL-13/IgE) responses. These findings suggest that MSSM-002, and perhaps other antiasthma formulas, may offer some clinical advantages over corticosteroids because they are less likely to increase the patient's susceptibility to infection. In addition, although IFN- γ levels were higher in the MSSM-002 group compared with the sham-treated group, they were not higher than in healthy controls. This type of immunoregulation may prove to be more beneficial than T_H1 cytokine (IFN- γ and IL-12) or T_H1 adjuvant therapy, which may cause undesirable inflammation because of higher-than-normal levels of T_H1 cytokines.⁴¹⁻⁴³

The pharmacologically active components of MSSM-002 have not yet been characterized. However, some of the herbs in this formula are reported to exhibit anti-inflammatory and antiallergic properties.¹⁵ Further research will be required to dissect the mechanisms of action of this formula. It is worth noting that, unlike some other "antiasthma" TCM formulas, MSSM-002 does not contain *Ma-Huang*, a source of ephedrine. Overdoses of this herb have been reported to cause central nervous system stimulatory activities, increased blood pressure, and heart palpitations.⁴⁴ Thus MSSM-002 may also prove to have an advantage over other formulas for long-term treatment of allergic asthma.

In conclusion, MSSM-002 suppressed antigen-induced AHR in this experimental model of asthma. This effect was accompanied by reduction of eosinophilic inflammation, mucus hypersecretion, and specific down-regulation of T_H2 responses. This study suggests that some Chinese herbal medicines, such as MSSM-002, may be useful for treatment of asthma and allergy and that experimental studies may be useful in selecting candidate antiasthma herbal formulas for future clinical evaluation.

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