Airway Pressure Recording

Mice were anesthetized intraperitoneally with pentobarbital (80 mg/kg), and a 16-gauge cannula was placed in the trachea. Mice were ventilated with a constant tidal volume of 0.2 mL at 120 breaths/min. Decamethonium bromide (25 mg/kg) was administered into the inferior vena cava to eliminate muscle movements. Airway pressure was measured using a pressure transducer via a port in the tracheal cannula. A stable baseline was recorded for at least 2 minutes, acetylcholine (50 μg/kg) was injected into the inferior vena cava, and the recording continued for at least 4 minutes. Airway responsiveness was defined as the time-integrated change in peak airway pressure.[1]

Bronchoalveolar Lavage Fluid Preparation and Cell Differential Counts

The left mainstem bronchus was occluded using forceps, the right lung was lavaged with 0.5 mL of cold Hanks balanced salt solution (without calcium or magnesium), and the bronchoalveolar lavage fluid was collected into chilled tubes. The fluid was centrifuged, and the supernatant was transferred and stored at $-80°$ for cytokine measurement by means of enzyme-linked immunosorbent assay. The cell pellet was resuspended in Hanks balanced salt solution, and slides were prepared by means of centrifugation of samples at 700 rpm for 7 minutes using a cytospin. Total cell numbers were determined using a hemocytometer. The slides were then stained with Diff-Quick (Dade Diagnostics of PR, Aguada, Puerto Rico), and cell differential counts were obtained by counting at least 500 cells per slide by means of light microscopy.

Lung Histologic Analysis

The left lower lobe was fixed in neutral-buffered formaldehyde, and 5-μm paraffin sections were stained with periodic acid–Schiff (PAS) for evaluation of goblet cells. Randomly chosen lung sections were examined in a blinded manner without preview. Mucus expression was calculated, adapting a previously published method,[2] by determining the percentage of PAS-positive epithelial cells (number of PAS-positive cells divided by the total epithelial cell number) in at least 6 randomly selected bronchioles per animal. Results are expressed as the mean percentage of PAS-positive cells per bronchiole for each group of mice. In addition, lung sections were stained with hematoxylin-eosin. Perivascular and peribronchial inflammation were graded using a standard scoring method (0=normal, 1=mild, 2=intermediate, and 3=severe).[3]

Quantitative Real-time Polymerase Chain Reaction

Real-time reverse transcription quantitative polymerase chain reaction was performed using a previously published SYBR green protocol using ABI7900 HT (Applied Biosystems, Foster City, California).[4] Each transcript in each sample was assayed 3 times, and the median threshold cycle was used to calculate the fold change values and control changes for each gene. Three housekeeping genes were used for global normalization in each experiment (β-actin, RPS11, and α-tubulin). Data validity by modeling of reaction efficiency and analysis of measurement precision was described previously.[5] Primer sequences for these reactions have been previously described.[6]

Table E1. Individual Herbs in MSSM-002 and Doses Used[a]

Material Medica (Ping Yin)	Pharmaceutical name	Human daily dosage of raw herb (g)	Human daily dosage of extract (g)	Mouse daily dosage of extract (mg)
Bai Shao (BS)	Radix Paeoniae Lactiflorae	9	1.20	6.0
Da Zao (DZ)	Fructus Zizyphi Jujubae	6	2.22	8.5
Dang Gui (DG)	Radix Angelicae sinensis	9	4.62	17.9
Gan Cao (GC)	Radix Glycyrrhizae Uralensis	3	0.56	2.2
Ge Gen (GG)	Radix Puerariae	9	1.89	7.3
Huang Qin (HQ)	Radix Scutellariae baicalensis	9	2.05	7.9
Jie Geng (JG)	Radix Platycodi grandiflori	6	3.16	12.2
Ku Shen (KS)	Radix Sophorae Flavescentis	9	1.38	5.4
Ling Zhi (LZ)	Ganoderma	20	4.2	4.2
Sheng Jiang (SJ)	Rhizoma Zingiberis	6	0.39	1.5
Ting Li Zi (TLZ)	Semen Descurainiae seulepidii	9	0.82	3.2
Xing Ren (XR)	Semen Pruni armeniacae	9	1.03	3.9
Zi Su	Folium Perillae Frutescentis	9	4.63	9.0
Zhen Zhu Mu (ZZM)	Concha Margaritifera	15	0.25	0.5
ASHMI		32	3.6	9.5
MSSM-002		128	19.4	73.4

[a] MSSM-002 is composed of 14 herbs, which are listed in Ping Yin and by pharmaceutical name. The doses of formulas for MSSM-002 and ASHMI are also listed.

Table E2. Effects of Individual Herbs of MSSM-002 on AHR and Eosinophilic Lung Inflammation

Herbs	% of APTI reduction Vs Sham*	% of Eosinophil reduction Vs Sham*
DZ	−43.97	−58.69
ZZM	−30.65	23.07
GC	−13.5%	17.3
DG	−10.10	58.26
SZ	0.90	−1.12
XR	25,69	33.27
SJ	19.57	31.17
BG	20.62	51
LZ	26.00	63.76
HQ	30.6	22.12
TLZ	31.55	4.27
JG	39.95	30.88
GG	43,43	41.94
KS	52.12	31.12
MSSM-002	58.14	47.65
ASHMI	60	63

Abbreviations: AHR, airway hyperresponsiveness; APTI, time-integrated change in peak airway pressure; BG, Bai-Sao and Gan-Cao (commonly used as a pair in traditional Chinese medicine).

Antigen-sensitized and antigen-challenged mice were treated with individual herbs of the MSSM-002 formula or with the entire formula. Percentage reduction in APTI (a measure of AHR) and bronchoalveolar lavage fluid eosinophils were calculated based on the values of nontreated antigen-sensitized and antigen-challenged mice (conalbumin mice). A minimum of 4 mice were included in each group. Minus indicates an increase in APTI or eosinophil percentage vs nontreated conalbumin mice. Complete herbal names are listed in Table E1.

* APTI values in treated conalbumin mice were not normalized by subtracting the APTI values in naive mice.

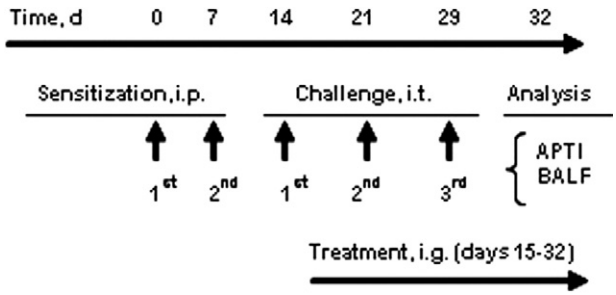

Figure E1. Experimental protocol. AKR mice (6 weeks old) were sensitized intraperitoneally (i.p) with concanavalin A (200 μg per mouse) twice at weekly intervals and then were challenged intratracheally (i.t.) on days 14, 21, and 29 with concanavalin A (100 μg per mouse). Twenty-four hours after the first challenge, mice were treated with 1 of the 14 herbs in MSSM-002 or with complete MSSM-002, all delivered at a dose of 1 mL per mouse intragastrically (i.g.) twice daily for 18 days. Nontreated antigen-sensitized and antigen-challenged mice and naive mice served as controls. Seventy-two hours after the final ovalbumin challenge, mice were humanely killed for analysis. APTI indicates time-integrated change in peak airway pressure; and BALF, bronchoalveolar lavage fluid.

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