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Anti-type I Allergic Mechanisms of *Mao-bushi-saishin-to* in Mice

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ABSTRACT—We investigated the anti-allergic effect of *mao-bushi-saishin-to* (MBS) on the type I allergy model in mice. When MBS was administered orally at a dose of 0.5 or 1.0 g/kg, edema of the footpad, the amount of plasma IgE and the ratio of eosinophilic leukocytes in peritoneal exudate cells were all dose-relatedly suppressed. Moreover to investigate the anti-type I allergic mechanisms of MBS, enzyme-linked immunosorbent assay was performed to determine the interleukin (IL)-4, IL-5 and interferon (IFN)-gamma production from splenocytes that were stimulated by pokeweed mitogen for 48 h. In addition, we assayed IgE production from splenic B cells stimulated with the lipopolysaccharide and IL-4 for 7 days. MBS inhibited the IL-4 and IFN-gamma production, but IL-5 and IgE production were not affected. Thus possibly, the inhibition of IL-4 production may partially be involved in the expression of the anti-type I allergic effects of MBS.

Keywords: *Mao-bushi-saishin-to*, Anti-allergy, Interleukin-4, IgE, Spleen cell

The herbal medicine *mao-bushi-saishin-to* {*Ma-Huang-Fu-Zi-Xi-Xin-Tang* in Chinese} (MBS), containing the three herb constituents of *mao* (Ephedrae Herba, Ephedraceae, *Ephedra sinica* STAPE), heat-processed *bushi* (Aconiti Tuber, Ranunculaceae, *Aconitum carmichaeli* DEBX.), called *shuchi-bushi* and *saishin* (Asiasari Radix, Aristolochiaceae, *Asiasarum heterotropoides* F. MAEKAWA *var. mandshuricum* F. MAEKAWA) in a ratio of 4:1:3, has long been prescribed for treatment of various inflammatory diseases (1). It is reported that MBS shows an anti type I allergic action against passive cutaneous anaphylaxis and analgesic activity on a pain model induced by chemical stimulation or physical stimulation (2, 3). Vascular permeability and the carrageenin edema were inhibited by suppressing chemical mediators such as histamine, bradykinin, leukotrienes and prostaglandins (4). Especially, IgE and cytokines such as interleukin (IL)-4 and IL-5 play important roles in type I allergic mechanisms. The IgE antibody response was regulated not only by antigen-specific helper and suppressor T cells, but also by isotype-specific soluble factors having an affinity for IgE (5 – 10). Moreover, recent studies have clearly indicated the presence of another IgE regulation pathway. Indeed, T cell-derived IL-4 and interferon (IFN)-gamma were found to have reciprocal activity on the regulation of IgE in mice as well as in humans (11 – 13). IL-4 plays an important role in the induction of IgE synthesis, whereas IFN-gamma suppresses IL-4-induced IgE synthesis. With regard to the T cell subsets responsible for the production of IL-4 and IFN-gamma, Mosmann et al. have reported that in the mouse system, $CD4^+$ T cell clones can be distinctly divided into two cell types (Th1 and Th2) based on the cytokine production pattern: IL-4 is produced by Th2 cells and IFN-gamma is produced by Th1 cells (14). We investigated the effect of MBS on type I allergy and examined the mechanism of the anti-allergic action by determining Th1, Th2 cytokine production and IgE production.

MATERIALS AND METHODS

Animals

Female Balb/c mice weighing 20 – 23 g were obtained at 5 weeks of age from Charles River Japan, Inc. (Kanagawa). The animals were housed in rooms kept at a temperature of 23 ± 2°C, a relative humidity of 55 ± 10% and a 12-h light – 12-h dark cycle and had free access to rodent chow (NMF; Oriental Yeast Co., Tokyo) and tap water.

Drugs and reagents

The MBS preparation used was a dry brown powder of the aqueous extract of *mao-bushi-saishin-to* (Tsumura & Co., Tokyo). In this study, drugs were suspended in a 5% aqueous solution of gum acacia (Wako Pure Chemical Industries, Ltd., Osaka) and given orally to animals after an overnight fast, at two dose levels of 0.5 and 1.0 g/kg in a constant volume of 10 ml/kg, respectively. Gum acacia

served as the control substance and was given in the same volume to animals in the control group. Prednisolone (Sigma Chemical Co., St. Louis, MO, USA) was used as a positive control medicine. Ovalbumin (OVA) (Sigma) and ragweed pollen extraction (Funakoshi, Tokyo) were used as antigen.

Immediate type allergic reaction

According to the procedure described by Inagaki et al. (15), OVA solution (0.5 mg/ml in saline) and $Al(OH)_3$ (Wako) (20 mg/ml in saline) were mixed in a 1 to 1 ratio, passed through a filter (0.45 μm), and then intraperitoneally administered at a dosage of 0.2 ml/mouse (sensitization). The second immunization was performed 20 days later. In addition, OVA solution (0.4 mg/ml in saline) (antigen challenge) was injected into a footpad (0.02 ml /mouse) 10 days later, and the thickness of foot paw was measured by a dial thickness gauge (Ozaki Mfg. Co., Ltd., Tokyo) antigen challenge after 15 min. Edema of footpad was calculated as the difference between measurements taken before and after the injection. The amount of IgE in the plasma was measured with an enzyme-liked immunosorbent assay (ELISA) kit (Yamasa Shoyu Co., Ltd., Tokyo). The medicine was administered orally for 30 days after the first sensitization until the assay date.

Eosinophilic leukocyte inducement with ragweed pollen

Using the method proposed by Kaneko et al. (16), 10% ragweed pollen extraction/saline (Torii Co., Tokyo) was administered to the murine dorsal subjacent skin (0.1 ml/mouse) on the first experimental day (day 0) and on days 1, 6, 8, 10 and 12. In addition, it was administered intraperitoneally on day 14. Twenty-four hours later, the number of eosinophilic leukocytes was counted and the results were expressed as the ratio of peritoneal exudate cells (PEC). MBS was administered orally from the first experimental day until the last day.

Measurement of cytokine production from spleen cells

Measurement of IL-4, IL-5 and IFN-gamma production from spleen cells was performed as previously described (17). Briefly, the spleen cells (2.5×10^6 cells/ml) from normal mice were suspended in 2% FBS/RPMI1640 culture medium, and the cells were cultured in the absence or presence of MBS (10, 30, 100 μg/ml) and pokeweed mitogen 5 μg/ml (Seikagaku Corporation, Tokyo) for 48 h at 37°C in a CO_2 incubator. The cytokines (IL-4, IL-5 and IFN-gamma) in the culture medium were measured with an ELISA kit (Amersham Japan Co., Tokyo).

Measurement of IgE production from splenic B cells

According to the procedure described by Snapper et al. (18), the nylon-adhering cells (B cell) were gathered from murine splenocytes and then cultured in RPMI1640 culture medium containing 10% FBS at the concentration of 2.5×10^5 cells/ml. MBS (10, 30, 100 μg/ml), rIL-4 (500 U/ml) (Pharmingen, San Diego, CA, USA) and lipopolysaccharide (LPS) (10 μg/ml) (Sigma) were added to the cells and cultured for seven days at 37°C in the CO_2 incubator. The IgE in the culture medium was measured with an ELISA kit.

Statistical analysis

The significance of differences was determined by one-way analysis of variance (ANOVA) followed by application of the Bonferroni/Dunn method. A value of $P<0.05$ was considered to indicate a statistically significant difference.

RESULTS

Influence on immediate type allergic reaction

The animals (control group) sensitized by the OVA solution showed an increased edema rate (54.4%) and higher level of IgE (502.7 ng/ml) compared to non-sensitized animals (normal group) (26.3% and 344.7 ng/ml, respectively). When MBS was orally administered to this sensitized model at a dose of 0.5 g/kg (41.6% and 406.4 ng/ml) and 1.0 g/kg (30.0% and 349.7 ng/ml), the increase in the edema rate and the amount of IgE were dose-dependently decreased. Moreover, administration of prednisolone at 0.001 g/kg suppressed the increase in the edema rate and the level of IgE (Figs. 1 and 2).

Influence on eosinophilic leukocyte inducement with ragweed pollen

The animals sensitized by ragweed pollen showed an increased ratio of eosinophilic leukocytes in PEC (44.7%) compared with the non sensitized animals (5.4%). When MBS was orally administered to this sensitized model at 0.5 g/kg (29.7%) and 1.0 g/kg (25.4%), the increase in the ratio was dose-dependently suppressed. Moreover, administration of prednisolone at 0.001 g/kg similarly suppressed the increase (Fig. 3).

Influence on cytokine production

When spleen cells were stimulated with pokeweed mitogen for 48 h, the production of IL-4, IL-5 and IFN-gamma were increased, respectively. MBS 10, 30 or 100 μg/ml suppressed the increase in IL-4 and IFN-gamma production dose-dependently. However, an effect on the increased IL-5 production was not recognized. Prednisolone at 0.01 μg/ml diminished the increase in IL-4, IL-5 and IFN-gamma (Table 1).

Influence on IgE production

B cells stimulated with LPS and rIL-4 for seven days

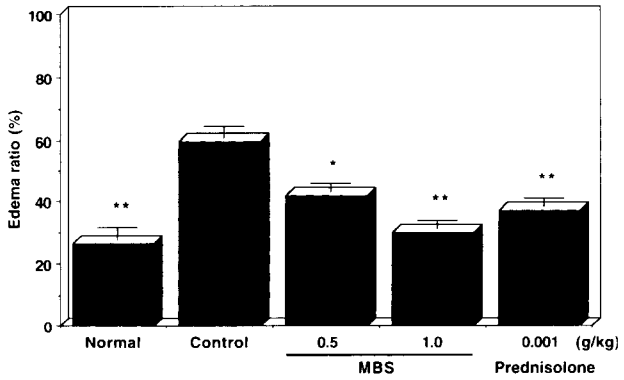

Fig. 1. Effects of MBS on OVA-induced edema of footpad in mice. The test was performed 1 h following the last administration of MBS at 0.5 g/kg, 1.0 g/kg or prednisolone at 0.001 g/kg (one time/day for 30 days). Each value represents the mean ± S.E.M. of 5 animals. Significantly different from the control group at *P<0.05, **P<0.01.

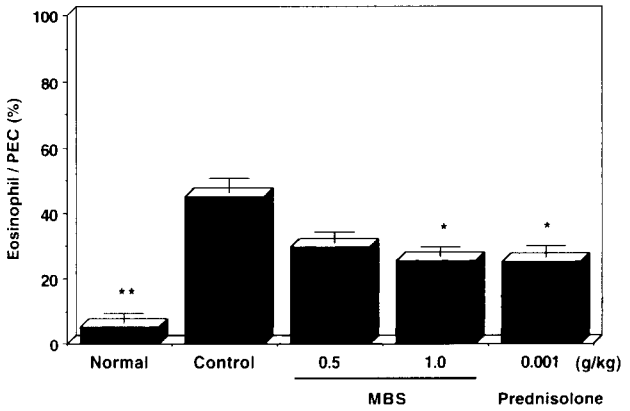

Fig. 3. Effects of MBS on ragweed pollen-induced eosinophil leukocytes in mice. The test was performed 1 h following the last administration of MBS at 0.5 g/kg, 1.0 g/kg or prednisolone at 0.001 g/kg (one time/day for 15 days). Each value represents the mean ± S.E.M. of 5 animals. Significantly different from the control group at *P<0.05, **P<0.01.

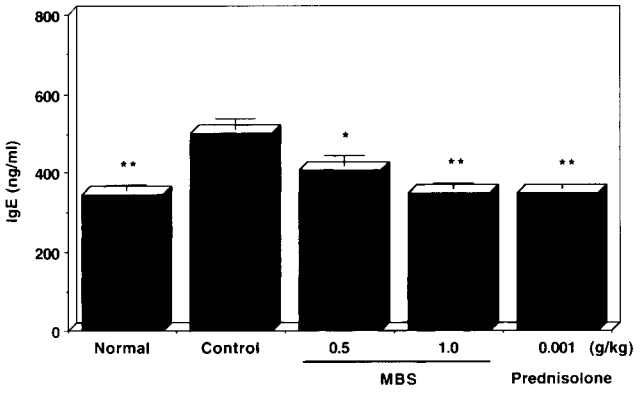

Fig. 2. Effects of MBS on OVA-induced IgE in the plasma of mice. The test was performed 1 h following the last administration of at MBS 0.5 g/kg, 1.0 g/kg or prednisolone at 0.001 g/kg (one time/day for 30 days). Each value represents the mean ± S.E.M. of 5 animals. Significantly different from the control group at *P<0.05, **P<0.01.

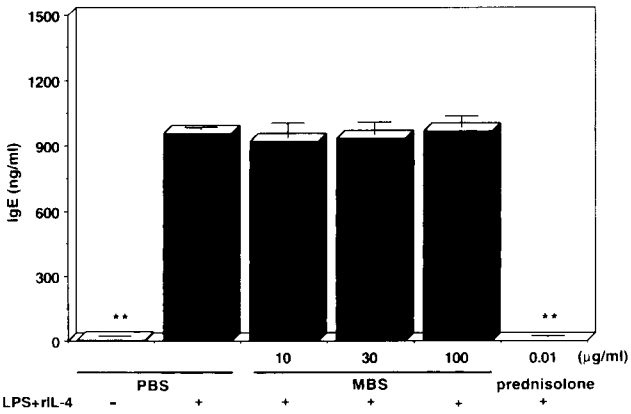

Fig. 4. Effects of MBS on LPS and rIL-4-induced IgE from murine B cells. MBS at 10, 30, 100 μg/ml, prednisolone at 10 ng/ml or PBS and rIL-4 (500 U/ml) or LPS (10 μg/ml) was added to the cells (2.5×10^5 cells/ml) and then cultured for seven days. Each value represents the mean ± S.E.M. of 5 wells. Significantly different from the PBS group at **P<0.01.

Table 1. Effects of MBS on IL-4, IL-5 or IFN-gamma production from murine splenocytes

Treatment	IL-4 (pg/ml)	IL-5 (pg/ml)	IFN-gamma (ng/ml)
None	18.0 ± 3.1**	10.4 ± 1.3**	4.9 ± 1.1**
Control	1071.2 ± 40.3	89.8 ± 15.8	23.4 ± 0.4
MBS 10 μg/ml	640.4 ± 39.4**	90.7 ± 10.8	23.9 ± 0.1
30 μg/ml	520.3 ± 59.9**	78.9 ± 6.0	21.7 ± 0.7
100 μg/ml	421.2 ± 20.7**	77.0 ± 12.7	9.4 ± 0.8**
Prednisolone 10 ng/ml	30.4 ± 3.6**	11.3 ± 2.1**	0.2 ± 0.1**

MBS at 10, 30, 100 μg/ml, prednisolone at 10 ng/ml or PBS and pokeweed mitogen at 5 μg/ml were added to the cells (2.5×10^6 cells/ml) and cultured for 48 h. Each value represents the mean ± S.E.M. of 5 wells. Significantly different from the control group at **P<0.01.

showed an increase in IgE production from 4.3 ng/ml to 960.0 ng/ml. MBS at 10, 30 and 100 μg/ml did not influence the increased production of IgE. Prednisolone at 0.01 μg/ml suppressed the increase in IgE (Fig. 4).

DISCUSSION

The incidence of allergic diseases such as bronchial asthma, rhinitis and atopic dermatitis has recently been increasing. The suppression of histamine is essential to anti-type I allergic effects. Several medicines such as tranilast and azelastine are clinically available for type I allergic patients. Most of such medicines directly inhibit the release of histamine from mast cells and eosinophils. Furthermore, Yanagihara et al. found that suplatast tosilate suppresses IgE antibody response and depresses the elevation of serum IgE levels (19). As a result, suplatast tosilate indirectly inhibits the release of histamine from mast cells and eosinophils. Their study indicated that suppression of IgE production was requisite to the expression of anti-type I allergic effects. We investigated the effects of MBS on footpad edema and plasma IgE levels in OVA-immunized mice. MBS diminished edema and inhibited IgE production. Alkaloids such as ephedrine contained in MBS are sympathomimetic agents and exert anti-inflammatory effect by inhibiting chemical mediators (20). MBS in this study was found to have an anti-inflammatory effect. To confirm whether this effect derives from the sympathomimetic effects of alkaloids, we investigated the anti-allergic rhinitis model using OVA-immunized mice. This model is generally thought to be related to IgE rather than sympathomimetic effects (21). To eliminate sympathomimetic effects of alkaloids, the administration of MBS was terminated 1 week before measurement of sneezing and plasma IgE levels. MBS suppressed sneezing and plasma IgE levels significantly (data not shown). These results suggest that the anti-type I allergic effect of MBS is not only due to the established effects of *mao* but due to the also suppression of IgE. Using cultured cells, we investigated the production of certain cytokines by splenocytes to clarify the anti-type I allergic mechanisms of MBS. MBS suppressed the increase in IL-4 production and IFN-gamma production from splenocytes. T cell-derived IL-4 plays an important role in the induction of IgE synthesis (11 – 13). Moreover, the reciprocal activity of IL-4 and IFN-gamma has been well documented both in mice and humans (11 – 13). These results show the suppression of IgE production by MBS may be due either to the inhibition of IL-4-induced IgE class switching at the B cell level or to the inhibition of IL-4 production at the T cell level. So, we investigated whether rIL-4-induced IgE production by LPS-activated B cells is suppressed by MBS. MBS did not exert any influence on the increased IgE production. Therefore, we speculated that MBS has no direct antagonistic action on IL-4. Moreover, we investigated the effect of MBS on infiltration of eosinophils and IL-5 production, which is thought to induce eosinophilic leukocyte proliferation (22). MBS inhibited the infiltration of eosinophilic leukocytes, but did not suppress the increase in IL-5 production from splenocytes. These results show that inhibition of eosinophil infiltration by MBS is not due to the suppression of IL-5 production. MBS may possibly inhibit eosinophil infiltration directly. Prednisolone depressed IL-4, IL-5 and IgE production. It was shown that the anti-type I allergic mechanism of MBS differed from that of prednisolone. Thus it is possible that the inhibition of IL-4 production may partially be involved in the expression of the anti-type I allergic effects of MBS. The inhibition of IL-4 production at the T cell level may accordingly be responsible for the IgE-suppressive activity of MBS (Fig. 5).

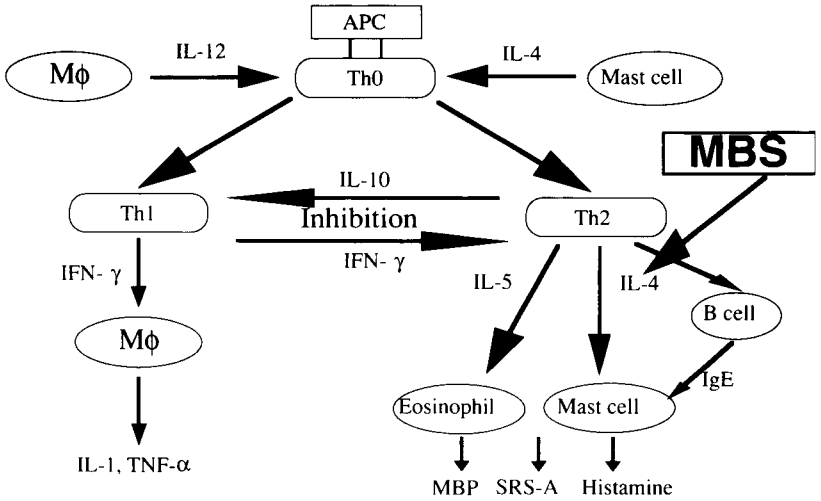

Fig. 5. A hypothetical model of cytokine regulation by MBS. Th1 and Th2 induce cellular and humoral immune responses, respectively. These two processes are cross-regulated by cytokines such as IL-4, IL-5 and IFN-gamma. The inhibition of IL-4 production from Th2 may partially be involved in the expression of the anti-type I allergic effects of MBS. APC: antigen presenting cell, MØ: macrophage, TNF: tumor necrosis factor, SRS-A: slow-reacting substance of anaphylaxis.

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