

Possible Involvement of Suppression of Th2 Differentiation in the Anti-allergic Effect of *Sho-seiryu-to* in Mice

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ABSTRACT—The clinical effectiveness of the *Kampo* medicine *Sho-seiryu-to* (SST) has recently been demonstrated in a double-blind randomized study of allergic asthma and rhinitis. We investigated the effect of SST on a type 1 allergic model in mice. Ovalbumin (OVA)-induced sneezing and the total and OVA-specific IgE levels were significantly suppressed with SST at 1.0 g/kg, but that of OVA-specific IgG_{2a} was not. In the splenocytes isolated from SST-administered mice, OVA-induced interleukin (IL)-4 production decreased while interferon (IFN)- γ production was not. The co-culture experiments using purified CD4⁺T cells and antigen-presenting cells (APCs) suggested that SST influenced both cell types. Flow-cytometric analysis showed that SST suppressed the number of IL-4 producing CD4⁺T cells but not the number of IFN- γ producing CD4⁺T cells. The CD86⁺ major histocompatibility complex class II⁺ (MHC II)⁺ cells and CD28⁺CD4⁺T cells were decreased by SST treatment, while CD80⁺MHC II⁺ cells, CD40⁺MHC II⁺ cells and CD154⁺CD4⁺T cells showed no change. These data suggested that SST may suppress IL-4 production in CD4⁺T cells via influencing CD28-CD86 interaction. In addition to the previously reported inhibitory activity on histamine release, suppression of Th2 differentiation at the stage of APC-CD4⁺T cell interaction may be involved in the anti-allergic effects of SST.

Keywords: *Sho-seiryu-to*, Th differentiation, Antigen-presenting cell, CD86, CD4⁺T cell

Anti-allergic drugs such as anti-histamines, anti-leukotrienes and inhibitors of other chemical mediators are frequently used to relieve the discomfort of allergic diseases (1). However, these drugs supposedly do not affect the induction phase of disease development, such as sensitization by antigen and the differentiation of type 2 helper CD4⁺T cells (Th2), because chemical mediators are released from the effector cells in the effector phase of the allergy. Antigen-presenting cells (APCs) phagocytize, process and present the antigen to CD4⁺T cells via major histocompatibility complex (MHC) molecules. The interaction between CD4⁺T cells and APCs requires signals mediated by MHC molecules (on APCs) and T cell receptors (on CD4⁺T cells), as well as co-stimulatory signals mediated by CD40, CD80 or CD86 (on APCs) and CD154 or CD28 (on CD4⁺T cells). The CD4⁺T cells, which have received information on the antigen through CD28-CD80 interaction, differentiate into Th1 cells (2, 3), while the CD4⁺T cells that interacted with CD86-expressing APCs

differentiate into Th2 cells (2, 3). Th1 cells, which produce interferon (IFN)- γ , interleukin (IL)-2 and tumor necrosis factor- α , evoke cell-mediated immunity and phagocyte-dependent inflammation (4). In contrast, Th2 cells, which produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13, evoke strong antibody responses (including those of the IgE class) and accumulation of eosinophils, but inhibit several functions of phagocytic cells (phagocyte-independent inflammation) (4). Therefore, inhibition of the polarization of the Th population toward the Th2 subtype may be an effective therapeutic strategy for allergic diseases. In fact, suplatast tosilate, which attenuates Th2 differentiation by suppressing the production of IL-4, has been reported to be beneficial in the treatment of various allergic diseases including asthma (5). However, it has not been reported whether suplatast tosilate affects APCs in APC-CD4⁺T cell interaction.

The *Kampo* medicine *Sho-seiryu-to* (SST, *Xiao-Qing-Long-Tang* in Chinese) has long been prescribed for the treatment of allergic diseases (6–8) and its clinical effectiveness was recently assessed in a double-blind randomized study for allergic asthma and rhinitis (9). SST has been

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reported to show an anti type 1 allergy action in passive cutaneous anaphylaxis in rats and in a nasal allergy model in ovalbumin (OVA)-sensitized guinea pigs (10). The suppressive activity by SST on chemical mediators, such as histamine and leukotrienes from peritoneal mast cells in vitro, has also been demonstrated (11). Thus SST has been suggested to have inhibitory effects on chemical mediators like many other anti-allergic drugs. However, the drug may have another mechanism of action, for example, via influencing APC-CD4⁺T cell interaction. Many Japanese herbal medicines including *Mao-bushi-saishin-to*, whose constituent herbs Ephedrae Herba (*mao*) and Asiasari Radix (*saishin*) are the same as those of SST, have been reported to have cytokine-modulating effects (12). Accordingly, in traditional therapy, SST has been used not only for curative treatment of allergic diseases but also for prevention. In the present study, we investigated the effect of SST on the interaction between APCs and CD4⁺T cells in a type 1 allergic model with mice sensitized by OVA. SST suppressed OVA-induced sneezing and the elevation of IgE levels. The effects of SST on Th1/Th2, the production of IgE, and the expression of co-stimulatory signals in APCs and CD4⁺T cells were also evaluated ex vivo using immune cells isolated from SST-administered animals.

MATERIALS AND METHODS

Animals

Female Balb/c mice weighing 20–23 g were obtained at 6 weeks of age from Charles River Japan, Inc. (Kanagawa). The animals were housed in rooms kept at a temperature of $23 \pm 2^\circ\text{C}$ and a relative humidity of $55 \pm 10\%$ under a 12-h light - dark cycle and had free access to rodent chow (NMF; Oriental Yeast Co., Tokyo) and tap water. All experiments were conducted according to the institution's guidelines for care and use of laboratory animals in research.

Drugs and reagents

The SST preparation used was a dry brown powder of the aqueous extract of *Sho-seiryu-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 at doses of 0.5 and 1.0 g/kg in a constant volume of 10 mL/kg. In all experiments, the medicine was administered orally once a day for 28 days. Gum acacia served as the control substance and was given in the same volume to animals in the control group. Because suplatast tosilate gave no beneficial results in the nasal allergy model, at least in the present experimental setting, prednisolone (Sigma Chemical Co., St. Louis, MO, USA) was used as a positive control. Ovalbumin (OVA, Sigma Chemical Co.) was used as an antigen.

Nasal allergic model and assay of total IgE, OVA-specific antibody

According to the procedure described by Watanabe et al. (13), who previously demonstrated the anti-allergic effect of SST, OVA solution (0.5 mg/mL in saline) and $\text{Al}(\text{OH})_3$ (Wako Pure Chemical Industries, Ltd.) (20 mg/mL in saline) were mixed and then intraperitoneally administered at a dosage of 0.2 mL/mouse (sensitization). A second and third immunization was given 7 and 14 days later. In addition, OVA solution (40 mg/mL in saline) (antigen challenge) was injected into the nostrils (0.02 mL/mouse) 21 to 28 days later and the number of sneezes was counted for 10 min after the last challenge (28 days). The total IgE in plasma, titer of OVA-specific IgE and IgG_{2a} in serum, were measured with an enzyme-linked immunosorbent assay (ELISA). Briefly, for the detection of OVA-specific IgE and IgG_{2a}, 96-well plates were coated with OVA (100 $\mu\text{g/mL}$), and bound serum antibodies were detected by biotinylated anti-mouse IgE or IgG_{2a} (Pharmingen, San Diego, CA, USA), followed by avidin peroxidase conjugate (Sigma). OVA-specific IgE and IgG_{2a} serum standards were obtained by pooling sera from OVA-sensitized mice. The 20-fold diluted OVA specific IgE and IgG_{2a} serum standards were decided in one unit. The OVA-specific antibody assay sensitivity was 0.0078–4 U/mL. The total IgE was measured with an ELISA kit (Yamasa Shoyu Co., Ltd., Tokyo). The assay sensitivity was 10–500 ng/mL. The samples of total IgE or OVA-specific IgE and IgG_{2a} were diluted.

Measurement of cytokine production by spleen cells or APCs and CD4⁺T cells

Levels of IL-4 and IFN- γ produced by spleen cells were measured as previously described (14). Briefly, the spleen cells (2.5×10^6 cells/mL) from normal or immunized mice were suspended in a 10% FBS/RPMI1640 culture medium and then cultured in the presence of OVA (1 mg/mL) for 72 h (IL-4 and IFN- γ) at 37°C in the CO_2 incubator. The APCs were prepared from splenic adherent cells and treated with 20 $\mu\text{g/mL}$ of mitomycin C (Kyowa Hakko Kogyo Co., Ltd., Tokyo) for 30 min. The purified adherent cells were found to comprise >86% APCs (CD11b⁺, B220⁺) by FACS analysis (Becton Dickinson, Mountain View, CA, USA). The CD4⁺T cells were prepared from splenic non-adherent cells. The splenic non-adherent cells were depleted of CD8⁺T cells, B cells and other accessory cells by treatment with a mixture of anti-CD8 Ab (Pharmingen), anti-MHC II Ab (Pharmingen), anti-CD24 Ab (Pharmingen) and anti-asialo GM1 Ab (Cedarlane Lab., Ltd., Ontario, Canada) on ice for 60 min, followed by complement-mediated lysis (rabbit complement from Cedarlane Lab., Ltd.) at 37°C for 45 min. The cell suspensions were depleted of residual B cells by adherence to goat anti-mouse Ig

(Pharmingen)-coated dishes for 60 min at room temperature. The purified T cells comprised >95% CD4⁺T cells as determined by FACS analysis. The APCs (1.25×10^6 cells/mL) and CD4⁺T cells (1.25×10^6 cells/mL) from normal or immunized mice were suspended in 10% FBS/RPMI1640 medium and cultured in the presence of OVA (1 mg/mL) for 96 h at 37°C in the CO₂ incubator. The levels of cytokines (IL-4 and IFN- γ) in the culture supernatant were measured with ELISA kits (Pharmingen). The culture supernatant of IFN- γ assay was diluted. The assay sensitivities were 15.625–2000 pg/mL (IL-4 system) and 78.125–5000 pg/mL (IFN- γ system).

IL-4 and IFN- γ secretion assays for CD4⁺T cells

To assay IL-4- and IFN- γ -producing cells in the CD4⁺T cells, the spleen cells isolated from normal or immunized mice were suspended in 10% FBS/RPMI1640 culture medium (at 2.5×10^6 cells/mL) and then cultured in the presence of OVA (1 mg/mL) for 72 h at 37°C in the CO₂ incubator. At the end of the culture, a portion of the cells was saved and analyzed using fluorescein isothiocyanate (FITC)-labeled anti-CD4 Ab (Pharmingen) and FACScan. The FITC⁺ cells were defined as the “total CD4⁺T cells”. The IL-4- or IFN- γ -producing cells were enriched with a MACS cytokine secretion assays kit (Miltenyi Biotec, Gladbach, Germany). Briefly, IL-4 or IFN- γ specific catch reagent (CD45 antibody conjugated to anti-cytokine antibody) was attached to the cell surface of the cultured spleen cells. The cells were then incubated for 45 min and the secreted IL-4 or IFN- γ bound to the IL-4 or IFN- γ catch reagent on the secreting cells. The caught IL-4 or IFN- γ was labeled with a second IL-4 or IFN- γ specific antibody, which was conjugated to phycoerythrin (PE). PE-labeled anti-IL-4 or IFN- γ antibody and FITC-labeled anti-CD4 Ab were added, and the cells were incubated on ice for an additional 15 min and then washed. The cells were magnetically labeled with anti-PE micro beads and enriched on a column that was placed in the magnetic field of a MACS separator. The cells were analyzed on a FACScan, and PE⁺ FITC⁺ cells in the enriched cells were defined as the “IL-4-“ or “IFN- γ -producing CD4⁺T cells”. The ratio of “IL-4-“ or “IFN- γ -producing CD4⁺T cells” to the “total CD4⁺T cells” was calculated.

Measurement of IgE production by splenic B cells

The splenic adherent cells were depleted of CD8⁺T cells, CD4⁺T cells and other accessory cells by treatment with a mixture of anti-Thy1.2 Ab (Pharmingen), anti-CD8 Ab, anti-CD4 Ab (Pharmingen), anti-CD11b Ab (Pharmingen), anti-CD11c Ab (Pharmingen) and anti-asialo GM1 Ab on ice for 60 min, followed by complement-mediated lysis at 37°C for 45 min. The cell suspensions were depleted of other residual cells using a 50%–70% percoll gradient

(Amersham Pharmacia Biotech UK Ltd., Tokyo). The purified B cells (the 50%–70% percoll interface) were >96% pure by FACS analysis and cultured in RPMI1640 medium containing 10% FBS at 2.5×10^5 cells/mL. Recombinant IL-4 at 250 U/mL (Pepro Tech, Inc., Rocky Hill, NJ, USA) and LPS at 10 μ g/mL (Difco Laboratories, Inc., Detroit, MI, USA) were added and the cells were cultured for 6 days at 37°C in the CO₂ incubator. The amount of IgE in the culture medium was measured with an ELISA kit. The culture supernatant of the IgE assay was diluted. The assay sensitivity was 15.625–1000 ng/mL.

Immunofluorescence staining

FITC-labeled anti-CD40 Ab (Pharmingen), anti-CD80 Ab (Pharmingen) or anti-CD86 Ab (Pharmingen) and PE-labeled anti-MHC II Ab (Pharmingen); FITC-labeled anti-CD4 Ab and PE-labeled anti-CD28 Ab (Pharmingen) or anti-CD154 Ab (Pharmingen) were added; and the spleen cells were incubated on ice for an additional 30 min and then washed. For each sample, 2.5×10^4 cells were analyzed on a FACScan.

Statistical analyses

The significance of the differences was determined by the application of the Dunnett method. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Influence on immediate type allergic reactions

The animals (control group) sensitized by the OVA solution showed increased sneezing (36.4 ± 1.03 counts/10 min) and an increased level of total IgE (1984.43 ± 443.57 ng/mL) compared with non sensitized animals (normal group) (5.0 ± 0.45 counts/10 min and 232.81 ± 71.99 ng/mL, respectively). When SST was orally administered to this sensitized model at a dose of 1.0 g/kg, the increase in the sneezing and the amount of total IgE were decreased (1.0 g/kg: 23.2 ± 2.58 counts/10 min and 489.77 ± 71.24 ng/mL, respectively) (Fig. 1: a and b). Further SST at a dose of 1.0 g/kg (IgE: 0.44 ± 0.13 U/mL, IgG_{2a}: 2.56 ± 1.62 U/mL) and prednisolone (IgE: 0.19 ± 0.04 U/mL, IgG_{2a}: 0.87 ± 0.47 U/mL) caused a decrease in OVA-specific IgE but did not change OVA-specific IgG_{2a} in comparison with the sensitized animals (IgE: 1.91 ± 0.64 U/mL, IgG_{2a}: 1.26 ± 0.61 U/mL) (Fig. 1: c and d).

Influence on cytokine production induced by OVA

When spleen cells of the control group were stimulated with OVA, the production of IL-4 significantly increased (869.91 ± 52.89 pg/mL) but that of IFN- γ did not increase (11107 ± 1036.65 pg/mL) in comparison with the non-

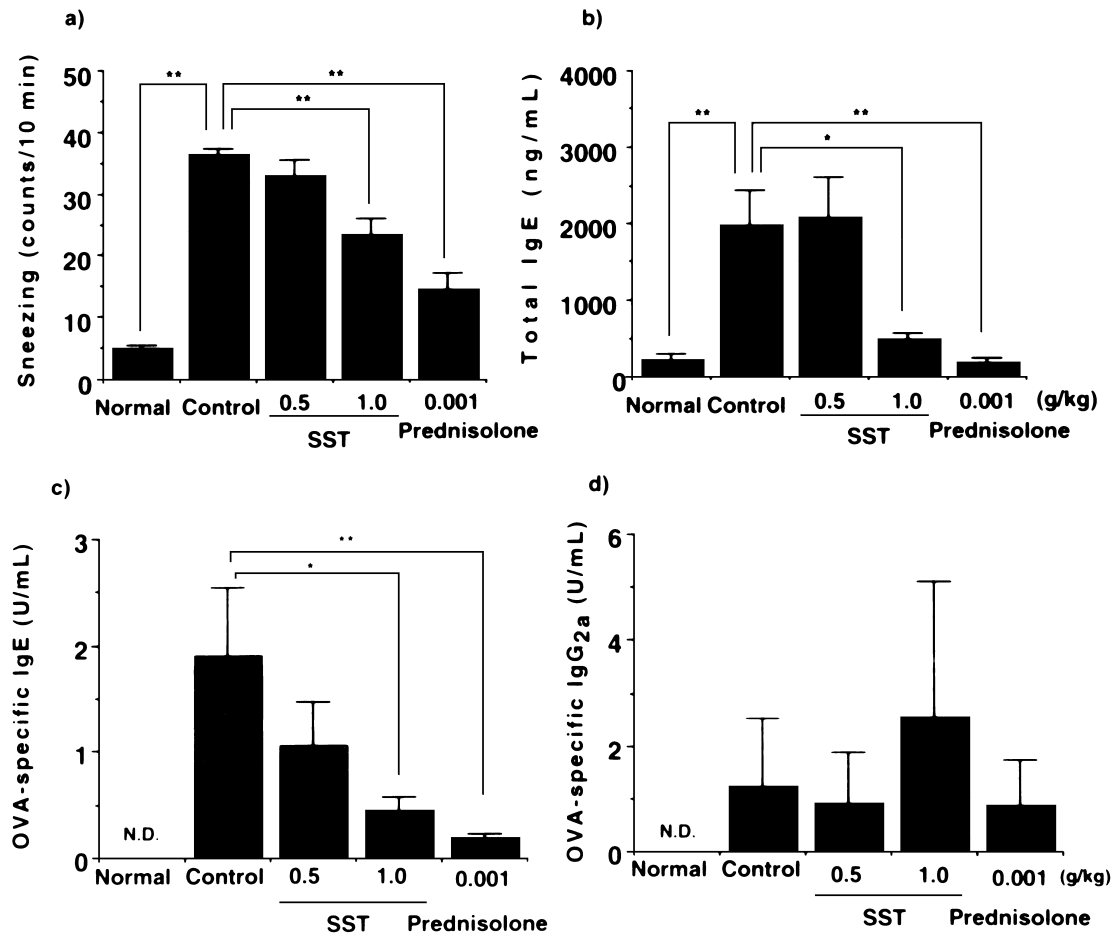


Fig. 1. Effects of SST on OVA-induced sneezing, total IgE and OVA-specific IgE or IgG_{2a}. The counts of sneezing and blood sampling were performed 1 h after the final administration of the vehicle, SST at 0.5 g/kg or 1.0 g/kg or else prednisolone at 0.001 g/kg (once/day for 28 days). The effect of prednisolone on this assay is shown for comparison. OVA-induced sneezing and the amount of total IgE are shown in panels (a) and (b), respectively. OVA-specific IgE and IgG_{2a} are shown in panels (c) and (d), respectively. Each value represents the mean ± S.E.M. of 5 animals. There is a significant difference between groups at * $P < 0.05$, ** $P < 0.01$.

sensitized (normal) group (IL-4: not detectable, IFN- γ : 9107.72 ± 859.25 pg/mL). Administration of SST suppressed the increase in IL-4 (1.0 g/kg: 583.12 ± 61.84 pg/mL) without markedly changing production of IFN- γ (1.0 g/kg: 12347.5 ± 878.41 pg/mL). Similar results were obtained for prednisolone (Fig. 2). When co-cultured with CD4⁺T cells from vehicle-treated mice, APCs from SST-treated mice resulted in a significant increase in IFN- γ production (2456.44 ± 235.17 pg/mL) in the culture supernatant relative to APCs from vehicle-treated mice (1598.52 ± 141.34 pg/mL) (Fig. 3: a and b). When co-cultured with APCs isolated from vehicle-treated mice, CD4⁺T cells from SST-treated mice resulted in significantly decreased IL-4 (1123.51 ± 37.26 pg/mL) and increased IFN- γ (2733.57 ± 106.11 pg/mL) levels compared to the CD4⁺T cells from vehicle-treated mice (IL-4: 1846.97 ± 41.62 pg/mL, IFN- γ : 1598.52 ± 141.34 pg/mL)

(Fig. 3: c and d).

Influence on IL-4- or IFN- γ -producing cells in CD4⁺T cells

When spleen cells of the sensitized mice were stimulated with OVA for 72 h, IL-4 producing cells in CD4⁺T cells increased in comparison with the non-sensitized mice (43.5% vs 1.28%). SST suppressed the increase of IL-4 producing CD4⁺T cells (15.8%). On the other hand IFN- γ -producing cells in CD4⁺T cells of the sensitized mice (3.96%) decreased in comparison with that of the non-sensitized mice (22.4%). That of SST-treated mice (5.61%) had no change in comparison with that of the sensitized mice. Similar results were obtained for prednisolone (Table 1).

Influence on IgE production

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

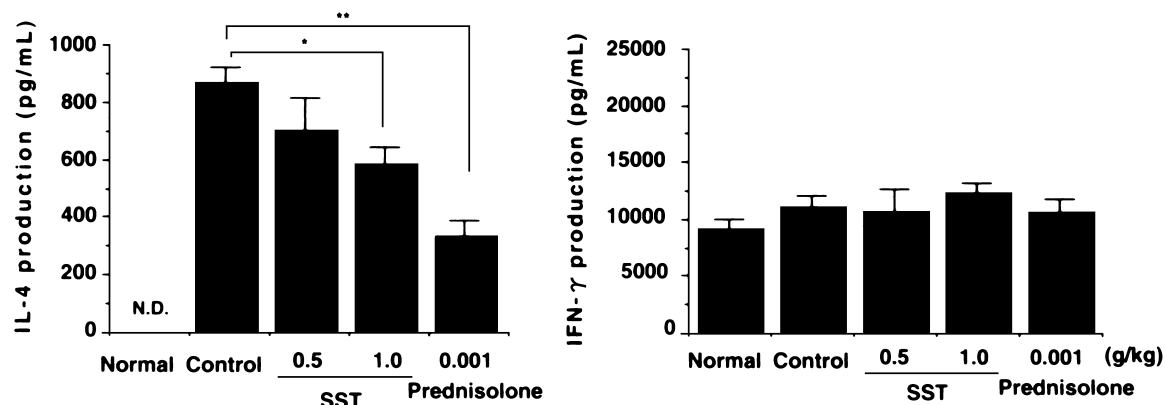


Fig. 2. Effects of SST on OVA-induced production of IL-4 and IFN- γ by murine splenocytes. One hour following the final administration of the vehicle, SST at 0.5 g/kg or 1.0 g/kg or else prednisolone at 0.001 g/kg (once/day for 28 days), spleen cells (2.5×10^6 cells/mL) were isolated from normal or immunized mice. The cells were cultured in 10% FBS/RPMI1640 medium in the presence of OVA (1 mg/mL) for 72 h at 37°C in a CO₂ incubator. Each value represents the mean $\pm$ S.E.M. of 5 animals. There is a significant difference between groups at * $P < 0.05$, ** $P < 0.01$.

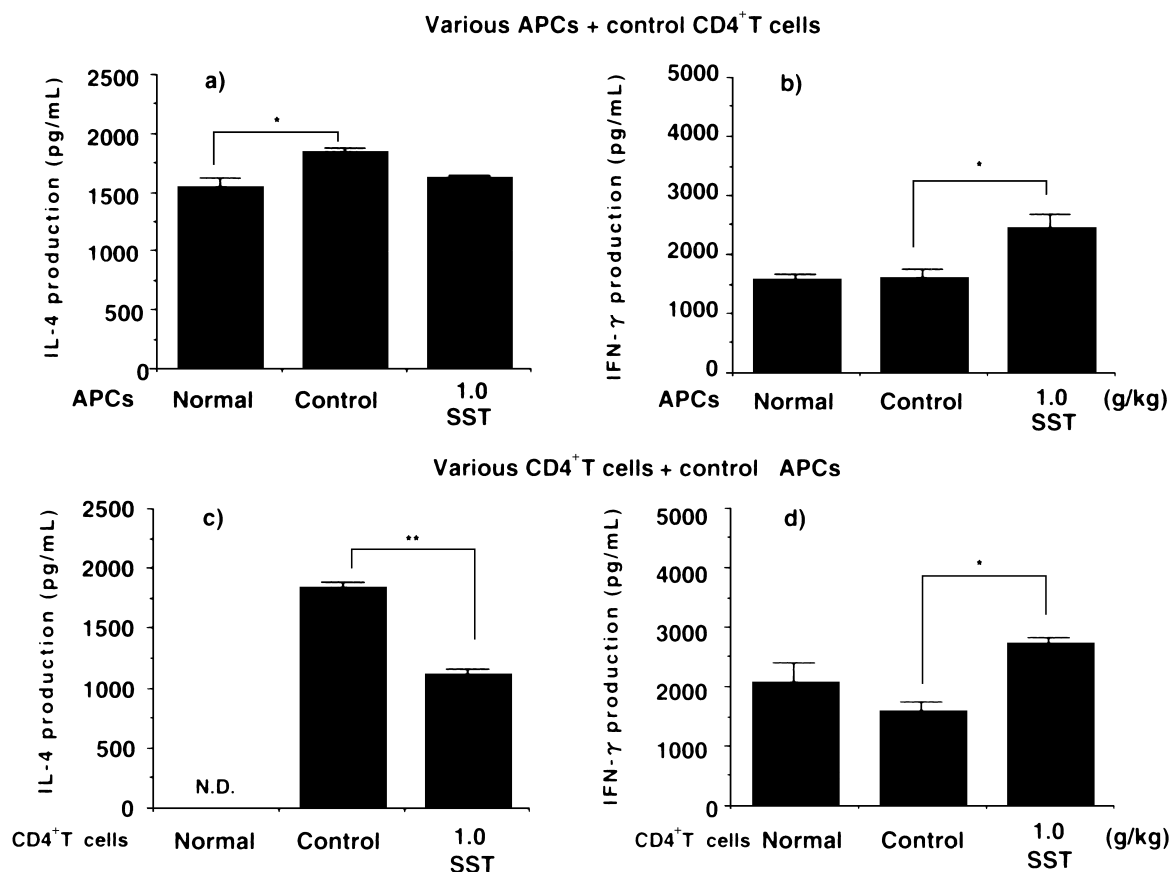


Fig. 3. Effects of SST on OVA-induced production of IL-4 and IFN- γ in co-cultures of murine APCs and CD4⁺T cells. One hour following the final administration of the vehicle or SST at 1.0 g/kg (once/day for 28 days), APCs (1.25×10^6 cells/mL) and CD4⁺T cells (1.25×10^6 cells/mL) were isolated from normal or immunized mice. The cells were cultured in 10% FBS/RPMI1640 medium in the presence of OVA (1 mg/mL) for 96 h at 37°C in a CO₂ incubator. IL-4 and IFN- γ levels in the supernatants of co-cultures of APCs from non-immunized, immunized or SST-administered immunized mice, and CD4⁺T cells from immunized mice are shown in panels (a) and (b), respectively. IL-4 and IFN- γ levels in the supernatants of co-cultures of CD4⁺T cells from non-immunized, immunized or SST-administered immunized mice, and APCs from immunized mice are shown in panels (c) and (d), respectively. Each value represents the mean $\pm$ S.E.M. for pooled APCs and CD4⁺T cells of 3 animals in triplicate cultures. Results are from one experiment repeated twice with similar results. There is a significant difference between groups at * $P < 0.05$, ** $P < 0.01$.

Table 1. Effects of SST on OVA-induced IL-4- or IFN- γ -producing CD4⁺T cells

Treatment	IL-4 producing cells	IFN- γ producing cells
	Cytokine producing CD4 ⁺ T cells/total CD4 ⁺ T cells (%)	
Normal	1.28	22.4
Control	43.5	3.96
SST (1.0 g/kg)	15.8	5.61
Prednisolone (0.001 g/kg)	10.4	4.46

One hour following the final administration of the vehicle or SST at 1.0 g/kg (once/day for 28 days), spleen cells (2.5×10^6 cells/mL) were isolated from normal or immunized mice. The cells were cultured in 10% FBS/RPMI1640 medium in the presence of OVA (1 mg/mL) for 72 h at 37°C in a CO₂ incubator. IL-4- or IFN- γ -producing cells were enriched using a MACS cytokine secretion assay kit. The cells before and after the enrichment were analyzed using FACSscan, and the ratio of the IL-4 or IFN- γ producing CD4⁺T cells to total CD4⁺T cells was calculated as described in Materials and Methods. Results are from one experiment repeated twice with similar results.

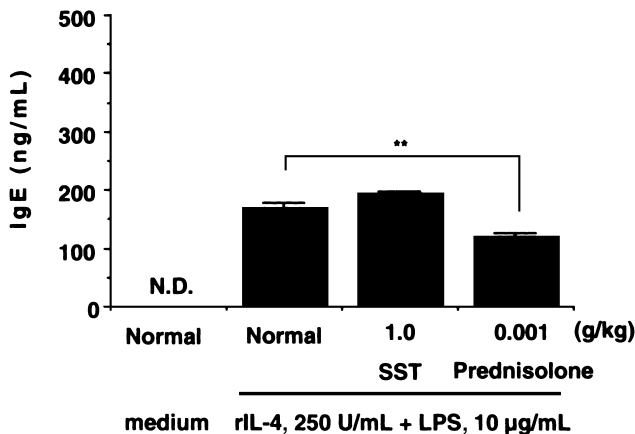


Fig. 4. Effect of SST on LPS and rIL-4-induced IgE production by murine B cells. One hour following the final administration of the vehicle, SST at 1.0 g/kg or prednisolone at 0.001 g/kg (once/day for 28 days), purified B cells (2.5×10^5 cells/mL) were isolated from normal mice. The cells were cultured in 10% FBS/RPMI1640 medium in the presence of rIL-4 (250 U/mL) and LPS (10 μ g/mL) for 6 days at 37°C in a CO₂ incubator. Each value represents the mean $\pm$ S.E.M. for pooled splenic B cells of 3 animals in triplicate cultures. Results are from one experiment repeated twice with similar results. There is a significant difference between groups at ** $P < 0.01$.

showed an increase in the production of IgE from undetectable levels to 170.86 ± 7.7 ng/mL. B cells isolated from SST-administered mice showed no change in the LPS/rIL-4-induced production of IgE. However prednisolone (1 mg/kg) suppressed the increase in IgE (Fig. 4).

Influence on the expression of co-stimulatory signals in spleen cells

APCs and CD4⁺T cells of the animals sensitized by OVA (control group) showed an increase in CD86⁺MHC II⁺ cells and CD28⁺CD4⁺T cells compared with those of non-sensitized animals (normal group). When SST was orally administered to this sensitized model at a dose of 1.0 g/kg, the

increases in CD86⁺MHC II⁺ cells and CD28⁺CD4⁺T cells were suppressed. However, neither CD80⁺MHC II⁺ cells, CD40⁺MHC II⁺ cells, nor CD154⁺CD4⁺T cells showed any difference among the groups. Prednisolone-administered mice suppressed all populations (Table 2, Fig. 5).

DISCUSSION

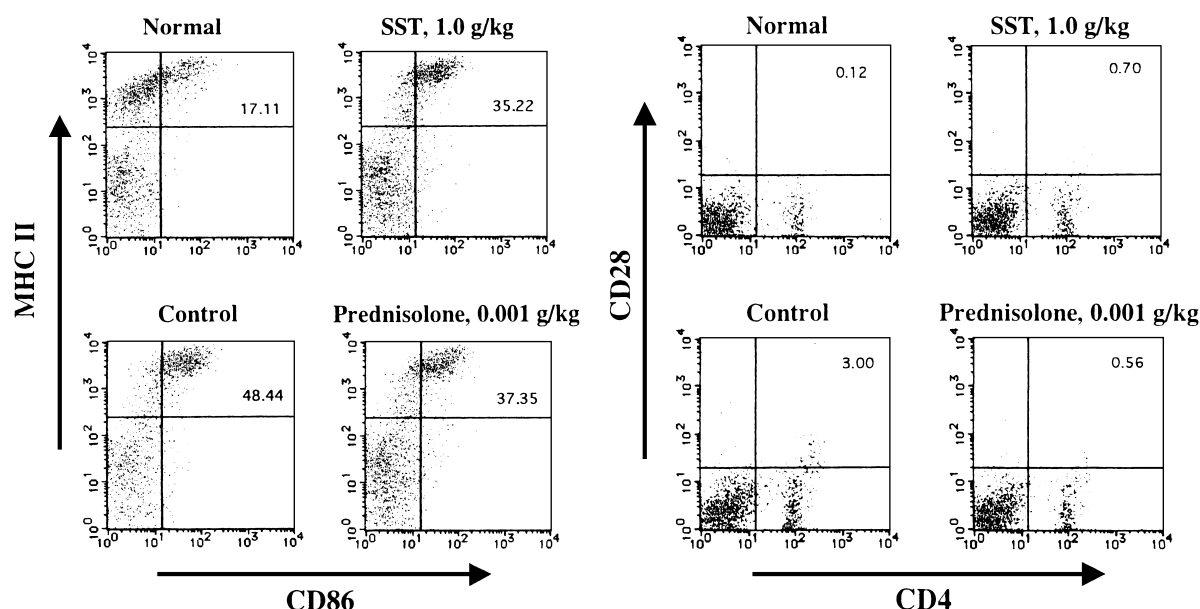
In the present study, we confirmed the dose-dependent anti-allergy effect of SST in sneezing and antigen-specific IgE levels in serum. The degree of suppression on the total IgE in plasma by the drugs (SST and the control drug prednisolone) did not coincide completely with the suppression of sneezing, while the degree of suppression on OVA-specific IgE apparently had a closer relationship. This is possibly because OVA-specific IgE is the primary cause of OVA-induced allergic reactions in this model. Furthermore, SST at 0.5 g/kg apparently suppressed OVA-specific IgE without any suppression on the total IgE, while prednisolone at 1 mg/kg suppressed the total IgE completely but OVA-specific IgE, incompletely. The results may be related to the difference in the mechanism between prednisolone and SST, where prednisolone had a wide range of immunosuppressive activity while SST may have had a more restricted effect. However, more extensive studies, including comparative analysis of dose-dependent anti-allergic effects by these drugs, are necessary to clarify this point.

There have been few studies on the possible effects of anti-allergic drugs on APC-CD4⁺T cell interaction because most of such drugs affect the function and release of histamine and other chemical mediators, which play important roles in the effector phase of type 1 allergic diseases (15). What we wish to show in this paper is that SST prevents nasal allergy by, at least partly, preventing or reversing the immunological and inflammatory process driving the allergy via the effect on APC-CD4⁺T cell interaction. Although SST has been known to have an inhibitory effect

Table 2. Effects of SST on the number of CD80⁺MHC II⁺ cells, CD86⁺MHC II⁺ cells, CD40⁺MHC II⁺ cells, CD28⁺CD4⁺ cells and CD154⁺CD4⁺ cells in spleen cells

Treatment	CD80 ⁺ MHC II ⁺	CD86 ⁺ MHC II ⁺	CD40 ⁺ MHC II ⁺	CD28 ⁺ CD4 ⁺	CD154 ⁺ CD4 ⁺
	(10 ⁶ cells/spleen cells)				
Normal	9.12 ± 0.31	17.82 ± 0.96**	29.03 ± 2.61	0.21 ± 0.06**	0.13 ± 0.01
Control	9.81 ± 0.63	48.14 ± 0.94	30.20 ± 2.38	1.21 ± 0.17	0.08 ± 0.02
SST (1.0 g/kg)	9.02 ± 1.38	37.06 ± 3.79*	35.80 ± 3.97	0.72 ± 0.12**	0.06 ± 0.02
Prednisolone (0.001 g/kg)	3.91 ± 0.26**	18.23 ± 1.52**	18.83 ± 2.04**	0.37 ± 0.04**	0.03 ± 0.01*

One hour following the final administration of the vehicle or SST at 1.0 g/kg (once/day for 28 days), spleen cells were isolated from normal or immunized mice. The cells were cultured in 10% FBS/RPMI1640 medium in the presence of OVA (1 mg/mL) for 72 h at 37°C in a CO₂ incubator. FITC-labeled anti-CD80 Ab (0.5 µg/mL), anti-CD86 Ab (0.5 µg/mL) or anti-CD40 Ab (0.5 µg/mL) and PE-labeled anti-MHC II Ab (0.25 µg/mL); FITC-labeled anti-CD4 Ab (0.5 µg/mL) and PE-labeled anti-CD28 Ab (0.5 µg/mL) or anti-CD154 Ab (0.5 µg/mL) were added, and the spleen cells were incubated on ice for an additional 30 min. Flow-cytometric analysis was performed using FACScan. Each value represents the mean ± S.E.M. of 3 or 5 animals. There is significant difference between groups at **P* < 0.05, ***P* < 0.01.

**Fig. 5.** Effects of SST on the population of CD86⁺ MHC II⁺ cells and CD28⁺CD4⁺ cells in spleen cells. One hour following the final administration of the vehicle or SST 1.0 g/kg (once/day for 28 days), spleen cells (2.5×10^6 cells/mL) were isolated from normal or immunized mice. The cells were cultured in 10% FBS/RPMI1640 medium in the presence of OVA (1 mg/mL) for 72 h at 37°C in a CO₂ incubator. FITC-labeled anti-CD86 Ab (0.5 µg/mL) and PE-labeled anti-MHC II Ab (0.25 µg/mL); FITC-labeled anti-CD4 Ab (0.5 µg/mL) and PE-labeled anti-CD28 Ab (0.5 µg/mL) were added, and the spleen cells (2.5×10^4 cells) were incubated on ice for an additional 30 min. Flow-cytometric analysis was performed using FACScan.

on histamine release from mast cells (16), the decrease in serum OVA-specific IgE and total IgE caused by SST cannot be explained by the above effect. Furthermore, the rIL-4- and LPS-induced IgE production by B cells isolated from SST-administered mice was no different from that by B cells isolated from vehicle-administered mice, while that by B cells isolated from prednisolone-administered mice was strongly inhibited. Therefore, we speculated that SST acts at the initial stage such as in the differentiation of Th1/Th2, and not at the stage of antibody production by acti-

vated-B cells. The investigation of the production of cytokines by splenocytes isolated from the mice administered vehicle or SST demonstrated that SST significantly suppressed an increase in IL-4 production, but not IFN- γ production. Accordingly, flow-cytometric analysis showed that SST decreased the IL-4 producing CD4⁺T cells but did not alter IFN- γ -producing CD4⁺T cells. These results suggest that SST may have selectively influenced the Th2 differentiation via suppression of IL-4 production. However, it is not plausible that SST inhibited IL-4 production

in CD4⁺T cells directly, because direct addition of SST into splenocyte culture produced no effect on IL-4 production (data not shown). Similar observations have been reported by Nagai et al. (17) using purified CD4⁺T cells. In addition, as demonstrated in Fig. 3, both APCs and CD4⁺T cells isolated from SST-administered mice exerted altered capacity to induce cytokine production; SST is therefore suggested to affect the APC-CD4⁺T cell interaction itself. The effect on the cell interaction has also been suggested by an analysis of co-stimulatory signals in APCs and CD4⁺T cells in OVA-stimulated splenocyte cultures. The number of CD86⁺MHC II⁺ cells or CD28⁺CD4⁺ cells was increased by OVA-sensitization, while that of CD80⁺MHC II⁺ cells, CD40⁺MHC II⁺ cells or CD154⁺CD4⁺ cells showed no significant change. These data suggested that the present allergic model was developed mainly via the CD28-CD86 co-stimulatory system, not by the CD28-CD80 nor CD40-CD154 systems. It has been reported that the expression of CD86 on APCs increased in type 1 allergy (18, 19). When APCs with a high level of CD86 expression interact with naive CD4⁺T cells (Th0 cells), the Th0 cells differentiate into Th2 cells and produce cytokines such as IL-4, which itself drives a shift in the Th1/Th2 balance toward Th2 (20, 21). These reports show that the CD28-CD86 system plays a critical role in the development of the Th2 response. SST significantly suppressed the increase of CD86⁺MHC II⁺ cells and CD28⁺CD4⁺ cells with no significant effect on the other populations, while prednisolone treatment decreased all populations. These data suggested that SST's suppressive effect on Th2 development in APC-CD4⁺T cell interaction is not due to overall immunosuppression. To clarify the mechanism by which SST exerts an anti-Th2 effect on APC-CD4⁺T cell interaction, a detailed investigation using active components and a well-defined in vitro system needs to be performed.

SST consists of eight herbs, *hange* (Pinelliae Tuber, Araceae, *Pinellia ternata* BREITENBACH), *kanzo* (Glycyrrhizae Radix, Leguminosae, *Glycyrrhiza uralensis* FISCHER), *keihi* (Cinnamomi Cortex, Lauraceae, *Cinnamomum cassia* BLUME), *gomishi* (Schisandrae Fructus, Schisandraceae, *Schisandra chinensis* BAILLON), *mao* (Ephedrae Herba, Ephedraceae, *Ephedra sinica* STAPF), *saishin* (Asiasari Radix, Aristolochiaceae, *Asiasarum heterotropoides* F. MAEKAWA var. *mandshuricum* F. MAEKAWA), *shakuyaku* (Paeoniae Radix, Paeoniaceae, *Paeonia lactiflora* PALLAS) and *kankyo* (Zingiberis Siccatum Rhizoma, Zingiberaceae, *Zingiber officinale* ROSCOE). We have previously reported that the *Kampo* medicine *Mao-bushi-saishin-to*, which contains *mao* and *saishin*, which are also present in SST, depressed an increase in IL-4 production by splenocytes in vitro (12). Thus *mao* and *saishin* may be involved in the decrease in IL-4 caused by SST. *Mao* contains certain alkaloids which have adrenergic

effects. Because noradrenaline has been known to modulate the balance of Th1/Th2 via the β_2 -adrenergic receptor on CD4⁺T cells (22, 23), the adrenergic action of *mao* alkaloids may contribute to the anti-Th2 effect of SST. However, extensive study is needed to clarify the active components and mechanisms responsible for the anti-allergic effects of SST.

The anti-Th2 action of SST during APC-CD4⁺T cell interaction apparently resembles that of suplastat tosilate, a newly developed anti-allergic drug capable of down-regulating the differentiation of Th2. The drug inhibits the production of Th2 cytokine by CD4⁺T cells, but its effect on APCs has yet to be reported. Although inhibitors of certain chemical mediators have been reported to influence Th2 cells and APCs, a detailed examination of their effect on APC-CD4⁺T cell interaction has not been performed. SST is a unique agent having inhibitory action against both the release of histamine and the development of the Th2 response at the stage of APC-CD4⁺T cell interaction. These results suggest that treatment with SST may not only be curative but also prophylactic for allergic diseases. Traditional *Kampo* therapy using SST, which aims at prevention of the onset/development of allergic diseases by long-term pre-treatment, may thus have some rationale.

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