Japanese traditional medicine, Senn-kinn-naidaku-sann up-regulates Toll-like receptor 4 and reduces murine allergic rhinitis*

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Abstract

Objective: To determine the mechanisms by which a traditional herbal medicine, Senkinnaidakusan (SKNS), controls Th2 responses, we examined the production of IL-12 by murine macrophages treated with SKNS.

Results: Treatment with SKNS significantly increased TLR4 mRNA in macrophages. Furthermore, pre-treatment with SKNS enhanced the production of IL-12 by macrophages stimulated with LPS. When SKNS was orally administered to C3H/HeN mice at the induction phase after OVA sensitization, the serum levels of OVA-specific immunoglobulin (Ig)E and IgG1 decreased, Interleukin (IL)-4 production by spleen T cells in response to OVA was significantly suppressed, while interferon (IFN)-γ production was increased. After nasal challenge of OVA, eosinophilic infiltration in the nasal mucosa and the number of sneezes were significantly inhibited in SKNS-treated mice compared with control mice. Besides, expression of IL-5 in the nasal mucosa was also inhibited. Using another strain of mice, C3H/HeJ (TLR4 negative), there was no difference in OVA-specific Igs or splenic cytokine production between the SKNS treatment and non-treatment groups. The eosinophilic infiltration in the nasal mucosa, the number of sneezes and IL-5 expression in the nasal mucosa were also not effected even after SKNS treatment.

Conclusion: These results suggest that oral administration of SKNS inhibits Th2 responses by enhancement of IL-12 release from macrophages via up-regulation of TLR4 expression.

Key words: Senn-kinn-naidaku-sann, allergic rhinitis, toll-like receptor, IL-12, macrophage

Introduction

Allergic rhinitis is an inflammatory disease associated with a Th2 response, airway infiltration by eosinophils, and nasal hyper-reactivity [1-5]. Naive clusters of differentiation (CD) 4+T cells initially stimulated with an allergen in the presence of interleukin (IL)-4 tend to develop into CD4+T cells, which secrete IL-4 and IL-13 for IgE isotype switching [6-8]. When the same allergen is inhaled, the allergen crosslinks IgE bound to high-affinity FcεR on mast cells lining the nasal mucosa, which consequently release stored mediators by granule exocytosis and synthesize leukotrienes and cytokines. Naive CD4+T cells that polarize into Th1 cells in the presence of IL-12, IL-15, IL-18 and IFN-γ, secrete IL-2, IFN-γ and tumor necrosis factor (TNF)-α not only for induction of cell-mediated immunity but also for down-regulation of Th2 responses [9-11]. Therefore, cytokines involved in Th1-biased response are thought to regulate Th2-mediated allergic response.

Many kinds of traditional Japanese herbal medicines have immunomodulating activities, e.g., B cell mitogenic activity [12], activation of macrophages [13], enhancement of natural killer (NK) cell activity (14) and actions on hematopoietic stem cells [15]. Senn-kinn-naidaku-sann (SKNS), a traditional Japanese medicine originating in China, is composed of 9 species of medicinal plants and is used for treatment of symptoms such

as general fatigue due to weakness and infection. However, the immunological mechanism of SKNS is not known. Recently, Buzhong-yi-qi-tang was reported to up-regulate Toll-like receptor (TLR)-4 expression on monocytes [16]. Toll-like receptors (TLRs) have been shown to play important roles in the recognition of bacterial components [17]. In mice, 10 members of the TLR family have so far been reported [18]. Among TLRs, TLR4 mediates LPS signal transduction in collaboration with other molecules, such as CD14, MD-2, myeloid differentiation factor 88 (MyD88), and Toll receptor-IL-1R domain-containing adaptor protein (TIRAP)/MyD88-adaptor-like (Mal) [17-20]. Traditional medicine, which is considered to be effective for infection, may influence TLR4 expression in macrophages and thereby on IL-12 production.

In this study, we examined the effects of SKNS on a murine allergic rhinitis model by enhancement of IL-12 production from macrophages via up-regulation of TLR-4 expression. We used C3H/HeN mice and C3H/HeJ mice, lacking the TLR-4 signaling pathway, to investigate the effect of SKNS on TLR-4.

Materials and methods

Mice

Female C3H/HeN and HeJ mice were obtained from Japan SLC (Hamamatsu, Japan) and used at 7–9 weeks of age.

Cells

All cell lines were grown in tissue culture flasks at 37°C in 5% CO_2/95% air and passaged every 2 or 3 days to maintain logarithmic growth. A mouse macrophage cell line, RAW264.7, was obtained from The Institute of Physical and Chemical Research cell bank (Tsukuba, Japan) and maintained in RPMI with 10% FCS (Sigma). Adherent cells from peritoneal exudates of naive C3H/HeN or C3H/HeJ mice were used as murine macrophages. Briefly, peritoneal exudates suspended in RPMI containing 10% FCS were cultured in plastic plates for 1 h at 37°C, non-adherent cells were removed, and fresh, complete medium was added to the adherent cells with or without stimulants.

Reagents and antibodies

Spray-dried SKNS was manufactured by Tsumura (Tokyo, Japan) as the test drug. SKNS was prepared as a hot water extracted from 9 species of medicinal plants, including Ginseng Radix 3g, Angelicae Radix 3g, Astragali Radix 3g, Cnidii Rhizoma 2g, Sinomeni Caulis et Rhizoma 2g, Platycodi Radix 2g, Magnoliae Cortex 2g, Angelicae Dahuricae Radix 1g and Glycyrrhizae Radix 1g. Extraction was carried out by boiling the mixture of herbs in 10 parts water at 95–100°C for 1 h, and the extract was spray-dried in a hot air stream [21]. LPS from *Escherichia coli* serotype B6:026, anisomycin, phorbol myristate acetate (PMA), dicoumarol, and p-nitrophenyl phosphate (pNPP) were obtained from Sigma Chemical Co. Concentrations of IL-4, IL-12 p40 and IFN-γ in the culture supernatants were measured by using commercial ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Anti-murine monoclonal IL-5 and IL-13 antibodies for use in Western blotting assay were purchased from Genzyme (Minneapolis, MA, USA).

Reverse transcription-PCR

Total cellular RNA from Raw264.7, HEK293 and mouse peritoneal macrophages was extracted with RNAzol B (Tel-Test, Friendswood, TX, USA) using a single-step isolation method [22] according to the manufacturer's instructions. RNase-free DNase (Takara Biochemicals, Shiga, Japan) was used to remove genomic DNA based on methods described previously [23]. Two micrograms of extracted RNA was reverse-transcribed into first-strand cDNA at 42°C for 40 min using 100 U/ml of reverse transcriptase (RT; Takara Biomedicals) and 0.1 μM oligo(dT) adapter primer (Takara Biomedicals) in a 50-μl reaction mixture. The TLR4 primers were 5′-GCA ATG TCT CTG GCA GGT GTA-3′ (sense primer) and 5′-CAA GGG ATA AGA ACG CTG AGA-3′ (antisense primer), yielding a product of 406 bp. Primers for murine -actin were 5′-TAA AAC GCA GCT CAG TAA CAG TCG G-3′ (sense primer) and 5′-TGC AAT CCT GTG GCA TCC ATG AAA C-3′ (antisense primer).

Western blot analysis

Proteins were obtained from the nasal mucosa of each mouse 12 hours after the final nasal challenge by using lysis buffer (50 mM Hepes, pH 7.0, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, 100 mM NaF, 10 mM NaPPi, 1 mM Na_3VO_4, 1 mM phenylmethanesulfonyl fluoride with aprotinin and leupeptin at 10 μg/ml). Samples were centrifuged (15000 rpm, 5 min), and the supernatants were stored at 80°C for further experiments. Lysates were analyzed on a 15% SDS-polyacrylamide gel, and the proteins were transferred to Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 1% bovine serum albumin in Tris-buffered saline containing 0.05% Tween-20 (TBST) for 1 h, and Western blot analysis was performed, followed by detection using an enhanced chemiluminescence system (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Immunization and treatment

Mice were each intraperitoneally immunized with 100 mg OVA absorbed on 100 ml of Alum on days 0 and 7. This was followed by daily intranasal (i.n.) challenge with 25 mg of OVA diluted by sterile normal saline from day 21 to day 28. In the SKNS treatment group, mice were orally administered with SKNS (suspended in phosphate-buffered saline (PBS)) or PBS everyday on days 1–8 using gastric tubes. Sera were obtained at 2-week intervals after immunization and stored at -20°C until analysis. Spleen cells were prepared 24 h after the last inhalation.

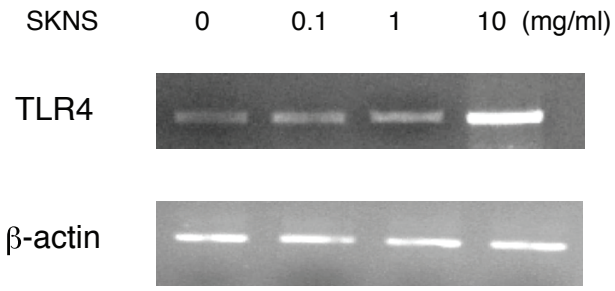

Figure 1. Gene expression of TLR4 in Raw264.7 cells after SKNS or LPS stimulation. Total RNA from Raw 264.7 cells stimulated with SKNS was reverse-transcribed with an oligo(dT) primer, followed by PCR with primers specific to TLR4 or to β-actin (as a loading control).

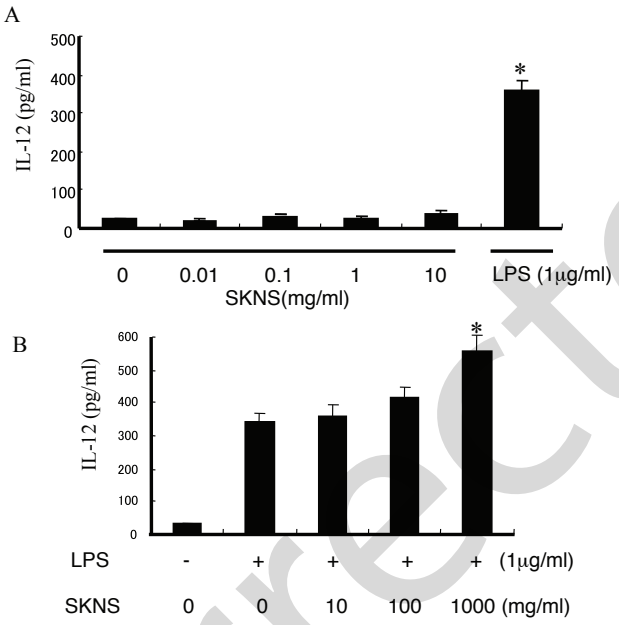

Figure 2. Effects of SKNS on IL-12 release from Raw264.7 cells.
A) Raw264.7 cells were incubated with SKNS (0.01-10 mg/ml). LPS was used as a positive control.
B) Raw264.7 cells were pre-incubated with SKNS (0.01-10 mg/ml) or PBS (control) for 30 min and then activated with LPS (1mg/mL). *p < 0.05.

Measurement of OVA-specific IgE, IgG1 and IgG2a

Levels of OVA-specific IgE, IgG1 and IgG2a were determined by ELISA. Sample wells of an ELISA plate were coated with OVA overnight and then blocked with 1% BSA in borate-buffered saline (0.05 M borate, 0.15 M NaCl, pH 8.6, 100 ml/well) at 37°C for 30 min. Diluted samples (100 ml/well) were incubated for 90 min at room temperature (Samples for IgE, IgG1 and IgG2a were diluted 1:100, 1:1,000 and 1:5, respectively). The plates were washed with borate-buffered saline with 0.05% Tween 20 and incubated with peroxidase-conjugated anti-mouse IgE, IgG1or IgG2a (Nordic Immunology) for 90 min at room temperature. After further washing, the plates were incubated for 20 min at room temperature with 100 ml/well of o-phenylendiamine solution (1 mg/ml with 3% H_2O_2), and OD was read at 492 nm [23,24].

Measurement of cytokine production by spleen cells

Spleen cells were incubated on a nylon wool column at 37°C in 5% CO_2 for 60 min. T cells (5×10^5) and MMC-treated naive splenocytes (5×10^5) were cultured in 96-well cell culture plates (Falcon, Becton Dickinson) with 200 mg OVA. After 48 h of culture, the cultured supernatants were collected and the amounts of secreted IL-4 and IFN-γ in the supernatants were determined by ELISA. For cellular proliferation, the cultures were pulsed with 1μmCi/well of [^{3}H]TdR for an additional 6 h after 48 h of culture. [^{3}H]TdR incorporation was determined by liquid scintillation counting [23,24].

Evaluation of nasal signs

Before the last i.n. challenge with OVA or PBS, mice were placed in an observation cage (one animal/cage) for about 10 min for acclimatization. After the i.n. challenge with OVA or PBS, mice were placed in the observation cage again and the number of sneezes were counted for 10 min by the method of Sugimoto et al. [25].

Histological examination

Mice were killed 12 hr after the last i.n. challenge with OVA or PBS. The heads were removed and fixed in 10% formaldehyde solution for 24 hr at room temperature. After fixation, the heads were decalcificated in 5% formic acid for 36 hr at room temperature and neutralized in 5% sodium sulfate solution for 12 hr at room temperature. Coronal nasal sections were then stained with hematoxylin and eosin, and the number of eosinophils in each side of the posterior edge of the nasal septum was counted microscopically using Image Pro Plus Imaging software (Media Cybernetics, Del Mar, CA, USA).

Statistical analysis

Statistical significance of the data was determined by Student's t-test. A value of $p < 0.05$ was taken as significant.

Results

Gene expression of TLR4 in Raw264.7 cells after SKNS treatment and LPS stimulation

We examined the expression of TLR4 mRNA in Raw264.7 cells. TLR4 gene expression was constitutively detected in Raw264.7 cells, and further increased with 10 mg /ml of SKNS-pretreatment. RT-PCR analysis of β-actin expression confirmed the

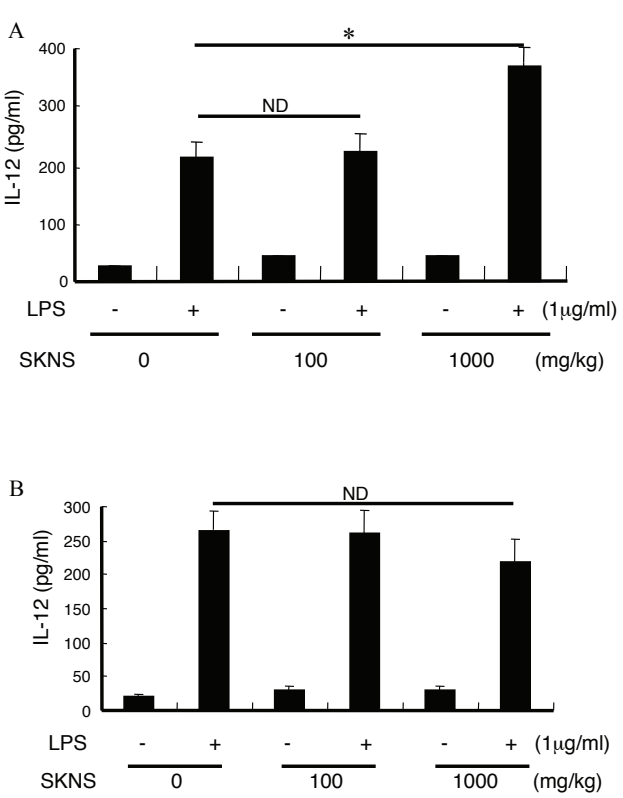

Figure 3. Effects of oral administration of SKNS on IL-12 release from peritoneal macrophages. A) Peritoneal macrophages derived from C3H/HeN mice orally given SKNS (1,000 mg/kg) were stimulated with LPS. After 24 h of culture, cell supernatants were harvested and analyzed for IL-12 by ELISA. Values are means ± SEM from three independent experiments and are expressed as nanograms per mL and 10^5 cells. *$p < 0.05$. B) Peritoneal macrophages derived from C3H/HeJ mice orally given SKNS (1,000 mg/kg) were stimulated with LPS. After 24 h of culture, cell supernatants were harvested and analyzed for IL-12 by ELISA. Values are means ± SEM from three independent experiments and are expressed as nanogram per mL and 10^5 cells. *$p < 0.05$.

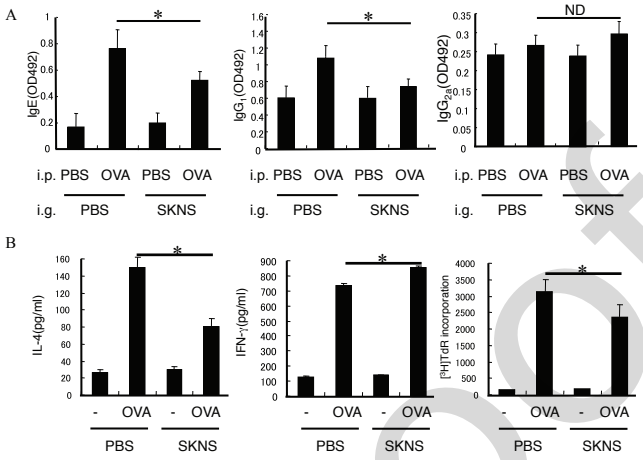

Figure 4. Production levels of OVA-specific Igs and cytokines in serum by splenic T cells. A) Serum levels of OVA-specific Igs in SKNS-treated and PBS-treated C3H/HeN mice in the eliciting phase. Data were obtained from three independent experiments and are expressed as means of triplicate determinations ± SD. *, $p < 0.05$; **, $p < 0.001$.
B) Cytokine production by splenic T cells from SKNS-treated and PBS-treated mice in the eliciting phase. Enriched T cells (5×10^5 cells) from the spleens of SKNS-treated and PBS-treated mice sensitized with OVA were cultured with or without OVA in the presence of MMC-treated spleen cells (5×10^5 cells) for 48 h at 37°C. All mice were immunized with OVA/ALUM. The proliferation activities of spleen T cells were assessed by incorporation of [^{3}H]TdR. IL-4 and IFN-γ production by T cells was assessed by ELISA. The data are representative of four independent experiments using pooled cells from three mice and are shown as means of triplicate determinations±S.D. *, $p < 0.05$; **, $p < 0.001$.

quality of all RNA preparations used for RT-PCR (Figure 1).

Induction of IL-12 production by Raw264.7 cells after SKNS or LPS stimulation

As shown in Figure 2A, a large amount of IL-12 is released from Raw264.7 cells after stimulation with LPS for 24 h, but SKNS stimulation alone had no effect on IL-12 production. On the other hand, when Raw 264.7 cells were prestimulated with 1,000 mg/ml of SKNS and LPS, IL-12 production was significantly increased (Figure2B).

Effect of SKNS treatment on IL-12 production by peritoneal macrophages stimulated with LPS

To determine the in vivo role of SKNS on IL-12 production by peritoneal macrophages, C3H/HeN and HeJ mice were orally administered with various doses of SKNS for 7 consequent days. After sacrificing the mice, peritoneal adherent cells including macrophages were collected. As shown in Figure 3A , IL-12 production by peritoneal macrophages derived from C3H/HeN mice that had been orally treated with SKNS at a dose of 1,000 mg/kg was significantly increased by stimulation with LPS in vitro. This result showed the effective dose of SKNS in vivo to be 1,000 mg/kg. On the other hand, oral treatment with SKNS did not influence IL-12 production by peritoneal macrophages derived from C3H/HeJmice, TLR4 gene mutant mice, following in vitro stimulation with LPS (Figure 3B). Thus, LPS seems to enhance IL-12 production by macrophages derived from C3H/HeJ mice via TLR4.

Impaired allergic rhinitis in C3H/HeN mice treated with SKNS

To determine which type of T cell response preferentially developed in mice treated with 1,000 mg/kg of SKNS in the induction phase by OVA sensitization, we assessed OVA-specific IgE/IgG2a production in the serum on day 14 after the first immunization with OVA/Alum. PBS-treated C3H/HeN mice had higher OVA-specific IgE and IgG1 levels in serum than did

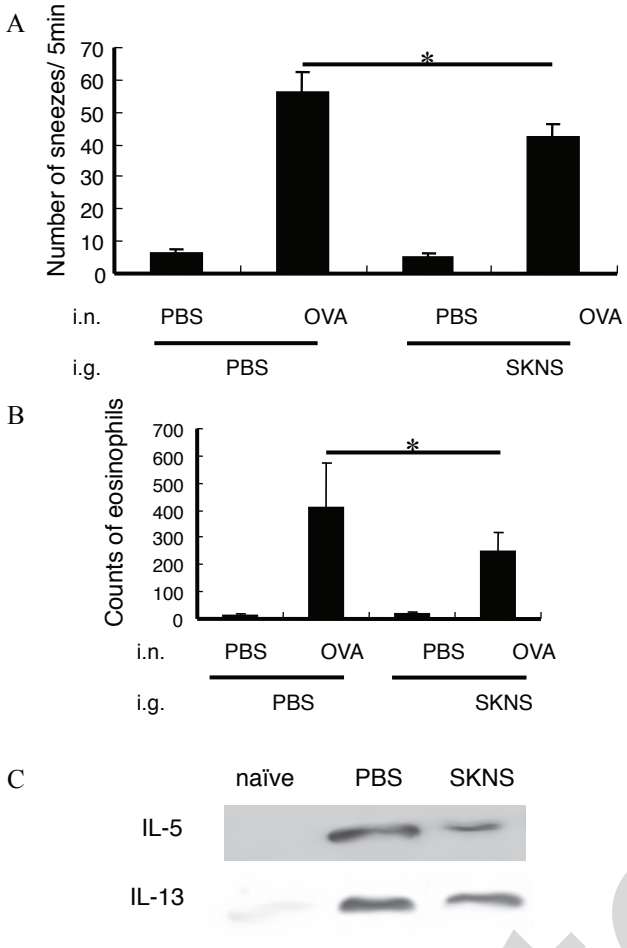

Figure 5. Allergic symptoms and eosinophil infiltration into the nasal mucosa following nasal challenge with OVA or PBS in SKNS-treated and PBS-treated C3H/HeN mice. A) OVA-sensitized mice were i.n. challenged with OVA or PBS on days 21-28 after the first immunization. After the last i.n. challenge with OVA or PBS , the numbers of sneezes were counted for 10 min.

B) The number of eosinophils infiltrating the nasal mucosa was counted microscopically. Mice were sacrificed 12 hr after the last nasal challenge with OVA or PBS. Coronal nasal sections were stained with hematoxylin and eosin. Data were obtained from three independent experiments, and results are geometric means ± SD. **, $p < 0.01$; ***, $p < 0.001$. C) Expressions of IL-5 and IL-13 were detected by Western blot analysis. Proteins were obtained from the nasal mucosa of each mouse 12 hours after the final nasal challenge using a lysis buffer.

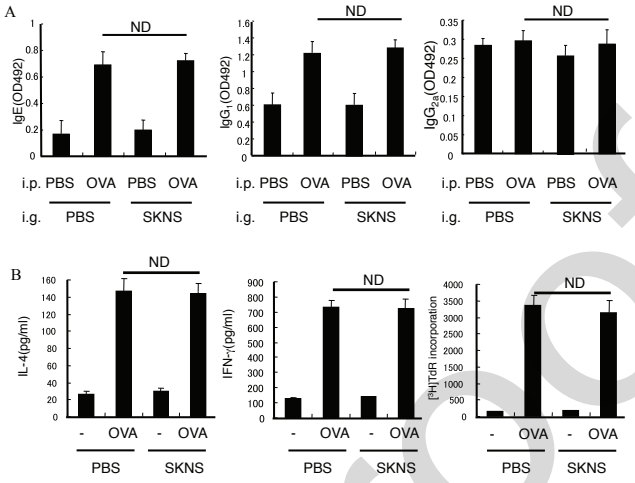

Figure 6. Production level of OVA-specific Igs and cytokines in serum by splenic T cells. A) Serum levels of OVA-specific Igs in SKNS-treated and PBS-treated C3H/HeJ mice in the eliciting phase. Levels of OVA-specific Igs were determined by ELISA in SKNS-treated and PBS-treated mice on days 0, 14, and 21. Data were obtained from three independent experiments and are expressed as means of triplicate determinations ± SD. *, $p < 0.05$; **, $p < 0.001$.

B) Cytokine production by splenic T cells from SKNS-treated and PBS-treated mice in the eliciting phase. Enriched T cells (5×10^5 cells) from the spleens of SKNS-treated and PBS-treated mice sensitized with OVA were cultured with or without OVA in the presence of MMC-treated spleen cells (5×10^5 cells) for 48 h at 37°C. All mice were immunized with OVA/Alum. The proliferation activities of splenic T cells were assessed by incorporation of [^{3}H]TdR. IL-4 and IFN-γ production by T cells was assessed by ELISA. The data are representative of four independent experiments using pooled cells from three mice and are shown as means of triplicate determinations ± SD. *, $p < 0.05$; **, $p < 0.001$.

SKNS-treated mice after OVA sensitization (Figure 4A). We then separated T cells from the spleens of mice that had been treated with SKNS or PBS after the last i.n. inhalation. As shown in Figure 4B, $CD3^+$T cells from PBS-treated C3H/HeN mice sensitized with OVA produced IL-4 in response to OVA, whereas IL-4 production was significantly reduced in SKNS-treated mice sensitized with OVA. IFN-γ production in response to OVA was increased in the culture supernatant of splenic T cells from SKNS-treated mice sensitized with OVA. We also examined the effects of SKNS on the murine nasal allergic phenomenon. The number of sneezes by each mouse over a 5 minute period after the last i.n. challenge with OVA or PBS was counted. The number of sneezes was significantly higher in PBS-treated mice than in SKNS-treated mice (Figure 5A). To investigate the effect of SKNS on the late-phase response in the effector phase, the number of eosinopils that had infiltrated the nasal mucosa was counted microscopically 12 hours after the last i.n. challenge with OVA or PBS. The infiltration of eosinophils into the nasal mucosa was aggravated in PBS-treated mice after the i.n. challenge with OVA compared with that in SKNS-treated mice (Figure 5B). Only a few eosinophils were detected in mice challenged with PBS. Next, we isolated nasal membrane proteins and investigated the expression of Th2-type cytokines by Western blot analysis. The expression of both IL-5 and IL-13 was significantly enhanced in PBS-treated mice following OVA inhalation compared with those in SKNS-treated mice (Figure 5C). IL-4 expression was not detected by

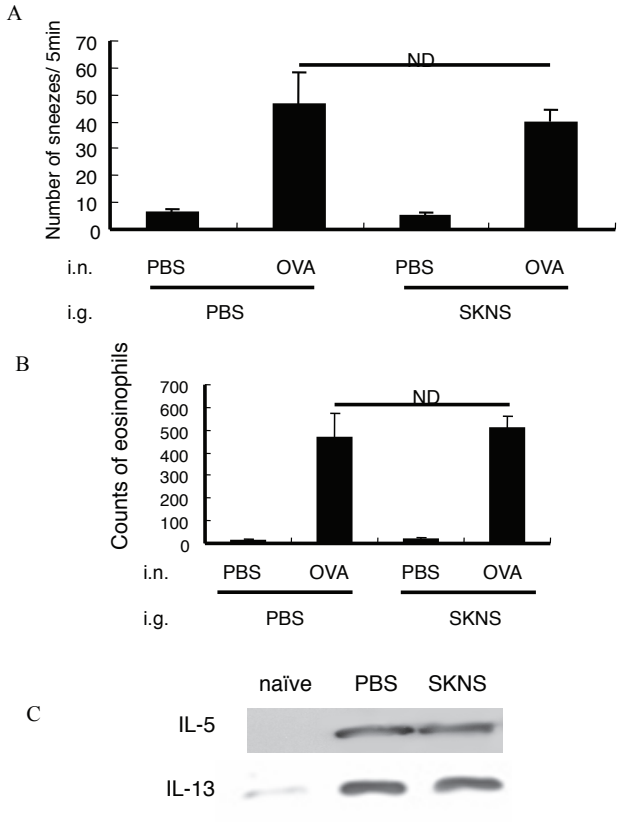

Figure 7. Allergic symptoms and eosinophil infiltration into the nasal mucosa following nasal challenge with OVA or PBS in SKNS-treated and PBS-treated C3H/HeJ mice. A) OVA-sensitized mice were i.n. challenged with OVA or PBS on days 21-28 after the first immunization. After the last i.n. challenge with OVA or PBS, the number of sneezes was counted for 10 min.

B) The number of eosinophils infiltrating the nasal mucosa was counted microscopically. Mice were sacrificed 12 hr after the last nasal challenge with OVA or PBS. Coronal nasal sections were stained with hematoxylin and eosin. Data were obtained from three independent experiments, and results are geometric means ± SD. **, $p < 0.01$; ***, $p < 0.001$.

C) Expressions of IL-5 and IL-13 were detected by Western blot analysis. Proteins were obtained from the nasal mucosa of each mouse 12 hours after the final nasal challenge by using a lysis buffer.

Western blot analysis.

No enhancement of allergic rhinitis by SKNS in C3H/HeJ mice

OVA-specific IgE and IgG1 levels in serum from SKNS-treated C3H/HeJ mice were not influenced by SKNS treatment (Figure 6A). There was also no difference between the level of IL-4 production or between the level of IFN-γ production by splenic T cells in SKNS-treated and PBS-treated C3H/HeJ mice (Figure 6B). Moreover, sneezing counts, eosinophilic infiltration and IL-5 expression in nasal mucosa did not decrease even after SKNS treatment (Figure 7A, 7B, and 7C).

Discussion

In this study, we assessed the efficacy of SKNS treatment in a murine allergic rhinitis model. SKNS is known from experience to be effective for treatment of infectious diseases, though the mechanism of action is not clear. Recently, it has been shown that Bu-zhong-yi-qi-tang, a traditional Japanese medicine, enhances the expression of TLR4 on human monocytes [16]. Traditional Japanese medicines that are effective for treatment of infectious diseases such as SKNS or Bu-zhong-yi-qi-tang may influence macrophages, particularly via up-regulation of TLR4 expression on macrophages. Since TLR4 mRNA is constitutively expressed in unstimulated macrophages, TLR4 must be essential for the initiation of innate immunity in the first encounter with Gram-negative bacteria that are present in the bowel [26]. Stimulation of macrophages via TLR4 enhances the production of various cytokines productions including IL-12 [27], IL-15 [26], IL-18 [28], IL-6 [29] and TNF-α [30]. IL-12 is essential for the polarisation of antigen-specific Th1 responses [31-34]. It is thought that up-regulation of Th1 responses inhibit Th2 responses and the development of atopic diseases [35-40]. We therefore focused on the role of SKNS in the expression of TLR4 on macrophages and the production of IL-12 by macrophages.

First, we assessed the effects of SKNS on the expression of TLR4 on murine macrophages. Surprisingly, it was found that SKNS alone enhanced the expression of TLR4 mRNA. SKNS did not directly influence IL-12 production by macrophages in vitro, though pretreatment with SKNS enhanced IL-12 production by macrophages following stimulation with LPS. Peritoneal macrophages derived from mice that had been orally administered SKNS produced a large amount of IL-12 following stimulation with LPS in vitro, although no effect on IL-12 production by peritoneal macrophages derived from C3H/HeJ mice, TLR4-gene mutant mice, was observed. SKNS is therefore thought to affect the expression of TLR4 mRNA on macrophages and enhance IL-12 production by macrophages stimulated with LPS.

Next, we assessed the effects of SKNS in a murine allergic rhinitis model. Oral treatment with SKNS successfully resulted in inhibition of OVA-specific IgE and IgG1 production in C3H/HeN mice. Besides, the production of IL-4 by splenic T cells derived from SKNS-treated C3H/HeN mice specific for OVA was significantly decreased compared with that in control mice. In contrast, there was no difference between production levels of OVA-specific serum Igs or splenic cytokines production in C3H/HeJ mice treated with SKNS and those not treated with SKNS. After nasal inhalation of OVA, numbers of sneezes, eosinophilic infiltration and IL-5 expression in nasal mucosa were significantly decreased in SKNS-treated C3H/HeN mice. However, no significant differences were seen in C3H/HeJ mice treated with SKNS and those not treated with SKNS. These findings indicate that SKNS has an

inhibitory effect in a murine allergic rhinitis model by enhancing IL-12 production from macrophages via TLR4.

Allergic rhinitis is a complex phenomenon driven predominantly by Th2-type cells [1-5]. Allergic rhinitis is characterized by overproduction of Th2 cytokines, which initiate and sustain allergic asthmatic inflammatory responses by enhancing the production of IgE and the growth [6], differentiation and recruitment of mast cells, basophils and eosinophils. Recently, it has been reported that treatment with various traditional medicines had a beneficial effect for patients with allergic rhinitis [41-43]. More recently, Mita reported that Bu-zhong-yi-qi-tang up-regulated TLR4 expression on macrophages [16]. Traditional medicines that are effective for treatment of infectious diseases may influence TLR4 expression on macrophages and enhance Th1 responses via up-regulation of IL-12 production. In the present study, we confirmed that SKNS up-regulates TLR4 gene expression on macrophages in vitro and in vivo. Furthermore, pretreatment with SKNS in vivo enhanced IL-12 production by macrophages following stimulation with LPS. Besides, SKNS inhibited Th2 responses and the allergic phenomenon in the murine allergic rhinitis model. In contrast, Th1 responses were up-regulated by SKNS treatment. The Th2 response is inhibited by IFN-γ-producing Th1 cells [44]. Clinical studies have demonstrated that reduced IFN-γ secretion in neonates is associated with subsequent development of atopy [45-47]. Furthermore, a predisposition toward the overproduction of Th1 cytokines may protect against atopy, because patients with multiple sclerosis [48], rheumatoid arthritis [49] or tuberculosis [50], conditions associated with increased production of Th1 cytokines, have a reduced predisposition toward the development of atopy. Macrophage/dendritic cell-derived cytokines such as IL-12, IL-15 and IL-18 are at least partly responsible for early IFN-γ production from NK and γδ T cells and, consequently Th1 cell differentiation. Alternatively, SKNS may contain ligands for NK and γδ T cells and directly stimulate the production of IFN-γ. Thus, the results of the present study suggest that methods to enhance IFN-γ production might be clinically useful in the prophylaxis of allergic rhinitis [44].

In summary, oral administration of SKNS inhibited Th2 responses in a murine allergic rhinitis model via up-regulation of TLR4 gene expression on macrophages. Our results thus offer a new approach using SKNS for the treatment of allergic disorders such as allergic rhinitis. Further studies are needed to elucidate the mechanisms of up-regulation of TLR4 gene expression on macrophages by SKNS.

Authorship contribution

Not available

Conflicts of Interest

None to report

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