Regulatory Effects of Gamisamul-tang on Atopic Dermatitis Induced in the NC/Nga Mice

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The present study was conducted to investigate the effect of Gamisamul-tang (GSMT) on atopic dermatitis (AD). AD was induced in NC/Nga mutant mice by DNCB treatment. GSMT administration reduced levels of skin severity scores. Serum levels of IgE, IgG, IgM, and inflammatory cytokines including IL-4, IL-4 and IL-13 were significantly decreased by GSMT treatment. Levels of mRNA's encoding IL-4, IL-6, IL-13, TNF-α, and interferon-γ in the dermal tissue and draining lymph node (DLN) by real time RT-PCR analysis showed decrease by GSMT testament. Moreover, the number of CD4+ and CD8+ cells was significantly decreased in the spleen and DLN tissues. Histological examination showed that infiltration levels of immune cells in ear, skin, and DLN of AD-induced NC/Nga mice were much improved by GSMT treatment. The present data suggest that GSMT may play an important role in recovering AD symptoms by regulating immune reactivity.

Key words : Gamisamul-tang, NC/Nga mouse, Atopic dermatitis, RT-PCR, inflammatory cytokine

Introduction

Atopic dermatitis (AD) is a chronic disease that affects the skin. In AD patients, the skin becomes extremely itchy and inflamed, causing redness, swelling, cracking, weeping, crusting, and scaling[1-4]. Atopic dermatitis most often affects infants and young children, but it can continue into adulthood or first show up later in life[3,6]. Atopic dermatitis is the most common of the many types of eczema. The cause of atopic dermatitis is not known, but the disease seems to result from a combination of genetic and environmental factors.

Although AD pathogenesis is largely unknown, it is evident that anaphylaxis hypersensitivity of immune reaction is most important for AD development. IgE-mediated immune reaction and involvement of inflammatory cytokines and histamine appears to be primarily important. Yet, molecular mediators in this immune response are also associated with other immune diseases such as asthma, rhinitis, and general allergic reactions. To investigate AD-specific pathogenesis, several animal models which include both spontaneous mutant and genetically engineered mice have been developed.

NC/Nga mouse has become much attention as animal model of AD in vivo. One feature of NC/Nga mice is that they can spontaneously develop dermatitis in the conventional (non-sterile), but not in the sterile pathogen free (SPF), maintenance condition[7]. This strain has been reported to have features such as a high susceptibility to X-irradiation and to anaphylactic shock induced by ovalbumin. Studies also show that allergic sensitivity is elevated by hapten treatment. For instance, significant skin changes were observed on the skin even where 2,4,6-trinitrochlorobenzene (TNCB) was applied in NC/Nga mice compared with wild type mice[8,9].

In Western medicine, anti-inflammatory drugs including steroid compounds such as corticosteroid and ceramide are generally used as AD therapy[10-12]. Acute effects of these medication are expected in many cases, but long-term cure is not guaranteed in most of the cases[13,14]. Oriental medicinal therapy has been one of major attention for AD treatment since potential side effects could possibly be minimized while expecting favorable treatment consequences. Yet, underlying mechanism on the effects of AD treatment have not been verified at cellular and molecular levels. In the present study, Gamisamul-tang (GSMT) was chosen to examine its effects on AD treatment using an experimental animal model NC/Nga mice. GSMT is used in the oriental medicine as an fortified

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prescription of Samultang for dermal problems and has been suggested to be effective for clearing the heat, palsy and detoxification. The present data showed that an administration of GSMT in AD-induced NC/Nga mice produced significant improvement of presenting AD development and favorable regulation of inflammatory cytokine related to hypersensitive immune responses in AD tissues.

Materials and Methods

1. Materials

1) Animals

Balb/C mice (4 weeks old obtained from KRIBB, Korea) and NC/Nga mice (6 weeks old, SLC, Japan) were fed food pellets (Samyang Co. Korea) and water ad libitum. The animals were adjusted for 2 weeks at 22±2℃ in a room of a relative humidity of 55±1.5%, light intensity of 150-300 Lux and 12 hr of electric light (07:00-19:00) and another 12 hr in the darkness. Only the healthy animals were selected for experiment.

2) Drugs

Gamisamul-tang (GSMT) used in this study was obtained from Daejeon University Oriental Hospital, and was purified before the experimental use. The recipe for 51 g of GSMT per a seal is composed of 5 g of Angelicae gigantis radix, 4 g of Lonicerae flos, Saururi herba seu rhizoma, Houttuyniae herba, Ulmus davidiana var. japonica, Puerariae radix, Aurantii immaturus fructus, and Rehmanniae radix preparat each, 3 g of Ledebouriellae radix, Paeoniae radix alba, Cnidii rhizoma, Tribuli fructus, 2 g of Polygoni multiflori radix, 1.5 g of Schizonepetae herba and Astragali radix, and 1 g of Glycyrrhizae radix. Two seals of GSMT were suspended in 2 liters of distilled water in an autoclave designed for the purpose of herbal drug exaction and boiled for 3 hr. GSMT filtrate was further concentrated by processing with a rotary vacuum evaporator (Büchi B-480 Co., Switzerland) and freeze-dried for 24 hr using the freeze-drier (EYELA, FDU-540, Japan). The yield was 18.4 g of GSMT powder from two seals (102 g) and the product was kept at -80℃ of deep freezer, and resuspended in distilled water immediately before use.

2. Induction of atopic dermatitis and drug treatment

The hairs on the back of NC/Nga mice at 11 weeks old were removed and maintained with no further treatment for 24 hr. 200 ㎕ of 1% DNCB solution (aceton:olive oil = 3:1) were treated on the skin, and 4 days later, 150 ㎕ of 0.2% DNCB solution were further treated 2-3 times a week and continued with the same treatment for 8 weeks. When the dermitis symptoms were severely developed before 8 weeks, DNCB treatment was stopped and used for the clinical test.

3. Clinical skin severity

The clinical severity of atopic dermatitis disorders were defined by classifying into 4 steps; 0 for none, 1 for mild, 2 for moderate, and 3 severe by evaluating the following 5 signals and symptoms including itch, erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness[6]. The symptoms were evaluated by observing skin dryness, eruption and injury on the body parts such as the ears, face, head and back. In case that the dermatitis is induced by DNCB treatment, 12-13 of the clinical score is regarded as a peak level of dermatitis.

4. Blood sampling, serum isolation, and measurement of IgG's and cytokines

Blood (100 ㎕) was collected using the capillary from the retro-orbital plexus under ether anesthesia of NC/Nga mice at 8, 12, 16, and 20 weeks of age and heparinized immediately thereafter. Plasma samples were obtained by centrifugation at 6500 rpm for 20 min. About 30 ㎕ of serum was collected and stored at -20℃ until use. IgE, IgG, and IgM levels were measured from serum by enzyme-linked immuno-sorbent assay kit (ELISA, Endogen, USA). Each antibody diluted with the coating buffer was coated on the surface of microplate at 4℃ overnight. Each well was washed three time with washing buffer and then 100 ㎕ of serum (100 fold diluted) was dispensed into the plate for 1 hr. Then the plate was washed twice, and 100 ㎕ of avidin-HRP conjugated antibody was treated, washed, and TMB solution was treated for 30 min in the dark. After stopping the reaction with 50 ㎕ of stop solution, the absorbance at 450 nm was measured by using ELISA reader. Spleen cells (2 x 10^6/mL) were cultured for 48 hr in the culture dish (Corning Inc, USA) which had been coated with anti-CD28 (1 μg/mL) and anti-CD3 (1 μg/mL). Then, IL-4 and IFN-γ levels were determined by using ELISA kit.

5. FACS analysis

1) Measurement of total immune cells

After erytherma induction with DNCB in NC/Nga mice (15 mg/25 g body weight, 20 weeks old), the mice were anesthetized with ethyl ether. Spleen and DLN were isolated and total immune cells were measured.

2) Immunofluorescence analysis of spleen and DLN cells

20 weeks after the induction of erytherma, spleen and draining lymph node tissues (DLN) were removed, and cells were separated using 100 mesh. Cells were washed twice after

5 min centrifugation at 1700 rpm with D-PBS and dissociated cells were selected by passing through the cell strainer (Falcon, USA) to remove cell debris and impure materials. Cells were treated with buffered ammonium chloride (ACK) solution containing 8.3 g of NH_4Cl and 1 g $KHCO_3$, in 1 liter of 0.1 ml EDTA solution at room temperature for 5 min to lyse erythrocytes, washed with D-PBS twice, and stained with 0.04 % tryphan blue to count cells. The number of splenocytes and lymph node cells was adjusted to 5×10^5 and used for immunofluorescence staining at 4℃. Antibody reaction was performed with FITC-anti-CD4 and FITC-anti-CD8 antibodies for 30 mins on ice. After the reaction, cell were washed with PBS at least three times and stained splenocytes and lymph node cells were analyzed by the flow cytometer and expression of antibody-positive cells were determined. $CD4^+$ or $CD8^+$ cells were analyzed by the Cell Quest Program (Becton Dikinson, USA)

6. RT-PCR

RNA was isolated from the skin and DLN tissues and RT-PCR analysis was performed essentially as described perviously[14]. Primers used for PCR are listed in Table 1.

Table 1. Primer sequences for real-time RT-PCR

mouse IL-6	sense	5' tccagttgccttcttgggac 3'
	antisense	5' gtgtaattaagcctccgacttg 3'
mouse IL-13	sense	5' atgcccaacaaagcagagac 3'
	antisense	5' tgagagaaccagggagctgt 3'
mouse TNF-α	sense	5' tgggaggaaaggggtctaag 3'
	antisense	5' acctacgacgtgggctacag 3'
mouse IL-4	sense	5' acaggagaagggacgccat 3'
	antisense	5' gaagccctacagacgagctca 3'
glyceraldehyde-3-phosphate dehydrogenase (G3PDH)	sense	5' tgcgctctagaaaaacctgccaa 3'
	antisense	5' gccccaggctcaaaggtg 3'
mouse interferon-γ	sense	5' tcaagtggcatagatgtggaagaa 3'
	antisense	5' tggctctgcaggatttttcatg 3'

7. Histological examination

Tissues from the left ear, DLN, and skin on the dorsal neck were removed and fixed in 10% paraformaldehyde solution for 24 hr. The tissues were embedded into the paraffin and 5 μm thickness of blocks were prepared. The sections were stained with haematoxylin and eosin (H&E) to differentiate inflammation and edema in epidermis, dermis, keratinocytes, neutrophils and eosinophils. The tissues were observed at 100x magnification using the phase contrast fluorescence microscope (Nikon, Japan).

8. Statistical analysis

The statistical data were represented as mean±standard error (SE), and the comparison amongst data was determined by Student's t-test.

Results

1. Effects of GSMT on clinical skin severity

Since GSMT treatment at 1-400 μg/mℓ on cultured mouse lung fibroblast cells (mLFCs) did not show any significant changes in cell survival, we began to examine the effect of GSMT on dermatitis in vivo by clinical skin severity scores. As shown in Fig. 1, saline-injected Balb/c mice did not show any clinical symptoms of dermatitis such as itch, erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness. GSMT administration into NC/Nga mice also did not show any distinct clinical skin symptoms compared with non-treated control by 12 weeks of age after DNCB treatment. Then, clinical manifestation of dermitis was rapidly developed between 13 and 17 weeks of age in NC/Nga control mice. Values of clinical skin severity were 10.9 ± 0.3 at 16 weeks and 11.8 ± 0.1 at 20 weeks in NC/Nga mice and 6.5 ± 1 at 16 weeks and 6.4 ± 0.9 at 20 weeks in GSMT-treated mice, indicating statistically significant decreases of clinical symptoms in GSMT animals compared with the control at 16 and 20 week time points respectively ($p<0.001$).

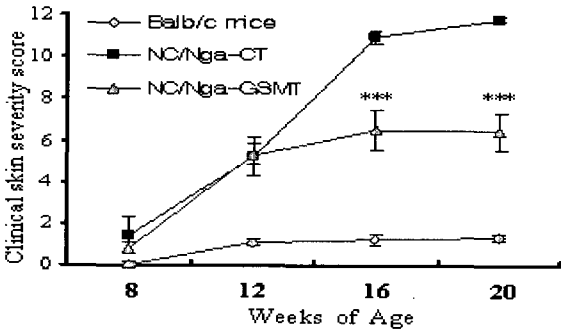

Fig. 1. Clinical skin severity score of DNCB-induced dermatitis in NC/Nga mice. Conventional female NC/Nga mice were administered with GSMT (15 mg/25g) for 8 weeks. A total clinical severity score for AD-like lesions was defined as the sum of the individual scores graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for each of five signs and symptoms (itch, erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness): Symptoms were evaluated by skin dryness, eruption and wound on the three parts of the body: ear, face and head, and back. (***$p<0.001$, Student's t-test).

2. Effects of GSMT treatment on immunoglobulin and cytokine levels in the serum of NC/Nga mice

Blood serum was collected from NC/Nga mice of 8, 16, and 20 weeks of age and used for IgE levels. Serum IgE levels of NC/Nga control mice were 7.6 ± 5.6 (pg/mℓ) at 8 weeks, 439 ± 105.7 (pg/mℓ) at 12 weeks, 4,811 ± 405.5 (pg/mℓ) at 16 weeks, and 5,645 ± 305 (pg/mℓ) at 20 weeks. In GSMT treated group, IgE levels were 7.9 ± 11 (pg/mℓ) at 8 weeks, 231.6 ±

37.6 (pg/㎖) at 12 weeks, 2111 ± 383.2 (㎍/㎖) at 16 weeks, and 3861 ± 718 (pg/㎖) at 20 weeks. The values at 16 and 20 weeks in GSMT group indicated significant decrease compared with those at the same time points of the control animals (Fig. 2). We further determined serum levels of other IgG molecules and cytokines at the time point of 20 weeks after administration. Serum IgG and IgM levels in NC/Nga mice were significantly lower in GSMT-treated animals than those in saline control (Table 2). Levels of IL-4, IL-5, IL-6, and IL-13 in the serum of GSMT-treated NC/Nga mice were significantly lower than those in the control group (Table 2). In contrast, serum IFN-γ levels were significantly elevated in GSMT treated animals compared with untreated control animals.

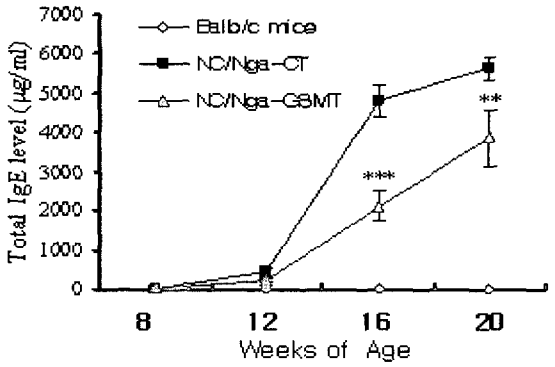

Fig. 2. Serum IgE levels in NC/Nga mice. NC/Nga mice was administered with GSMT (15 mg/25 g of body weight) for 8 weeks. Blood was collected from the retro-orbital plexus under ether anesthesia and heparinized immediately thereafter. Plasma samples were obtained by centrifugation and stored at -20°C until use. Total IgE levels were measured by a sandwich ELISA using an ELISA kit (Boehringer Mannheim Biochemical, Mannheim, Germany). Each point represents mean+SE (n=4). **p<0.01, ***p<0.001 (Student's t-test).

Table 2. Immunoglobulin and inflammatory cytokine levels in the serum of NC/Nga mice

	NC/Nga control	NC/Nga-GSMT
IgG	5494 ± 478 ㎍/ml	*3875 ± 694 ㎍/ml
IgM	1020 ± 105 ㎍/ml	**754 ± 75 ㎍/ml
IL-4	185 ± 48 pg/ml	*102 ± 42 pg/ml
IL-5	2,451 ± 211 pg/ml	**1652 ± 126 pg/ml
IL-6	1765 ± 287 pg/ml	*875 ± 331 pg/ml
IL-13	3,784 ± 362 pg/ml	**2,020 ± 506 pg/ml
IFN-γ	1,457 ± 240 pg/ml	*2230 ± 301 pg/ml

(*p<0.05, **p<0.01, Student's t-test)

3. Effects of GSMT treatment on cell numbers in the spleen and DLN

Spleen and DLN were isolated from NC/Nga mice which had been administrated with GSMT (15 mg/25 g) for 8 weeks, and used for immune cell analysis. In the spleen, total immune cell number was 19.13 ± 1.63 ($x10^7$) in NC/Nga control animal and 11.5 ± 1.5 ($x10^7$) in GSMT-treated animals, which showed significant difference between two groups ($p<0.01$) (Fig. 3A). Total immune cell number in DLN was 13.7 ± 1.10 ($x10^6$) in NC/Nga control animal and 7.5 ± 2.1 ($x10^6$) from GSMT-treated animals, indicating a significant decrease compared with the control group ($p<0.05$) (Fig. 3B).

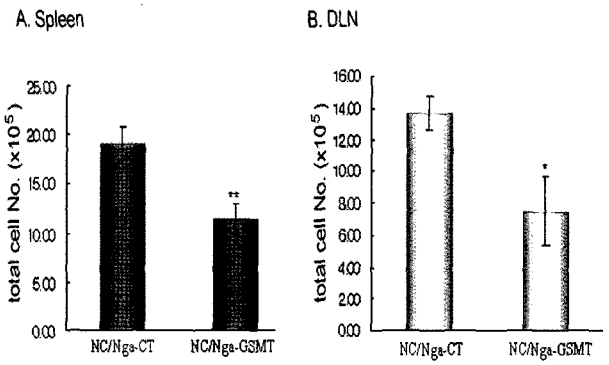

Fig. 3. Changes of total cell number in the spleen and DLN of NC/Nga mice. Mice were administered with GSMT (15 mg/25g) for 8 weeks. Total cell number of spleen or DLN were determined. Each point in (A) and (B) represents the mean+SE (n = 4). *p<0.01, **p<0.01, (Student's t-test).

4. Effects of GSMT on $CD4^+$, $CD8^+$ or NK^+ cell numbers

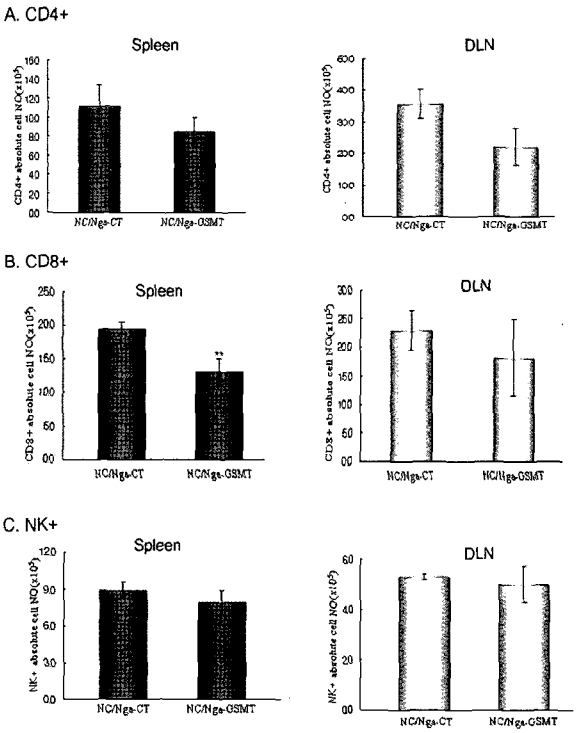

Fig. 4. Effect of GSMT extract on $CD4^+$, $CD8^+$, and NK^+ cells in spleen and DLN of NC/Nga mice. NC/Nga mice were administered with GSMT (15 mg/25g) for 8 weeks. Mouse spleen and draining lymph node were removed and isolated cells were used for flow cytometry. Each point represents the mean+SE (n=4). **p<0.01, (Student's t-test).

To determine $CD4^+$, $CD8^+$, and NK^+ cell population in the spleen and DLN, cultured cells were prepared and used for flow cytometry. $CD4^+$ spleen cells were 11.2 ± 2.2 ($x10^5$) in the control group and 8.5 ± 2.5 ($x10^5$) in GSMT-treated group.

$CD4^+$ DLN cells were 35.7 ± 4.6 ($x10^4$) in the control group and 22.2 ± 5.6 ($x10^4$) in the GSMT-treated group (Fig. 4A). $CD8^+$ spleen cells were 19.5 ± 0.8 ($x10^5$) in the control group and 13.1 ± 1.9 ($x10^5$) in GSMT-treated group showing significant decrease compared with the control group ($p<0.01$). $CD8^+$ DLN cells were 23.0 ± 3.5 ($x10^4$) in the control group and 18.2 ± 6.7 ($x10^4$) in the GSMT-treated group (Fig. 4B). NK^+ spleen cells were 8.9 ± 0.7 ($x10^5$) in the control group and 7.9 ± 1.0 ($x10^5$) in GSMT-treated group. In DLN, NK^+ cells were 5.3 ± 0.1 ($x10^4$) in the control group and 5.0 ± 0.7 ($x10^4$) in the GSMT-treated group (Fig. 4C).

5. Effects of GSMT on mRNA expression

RNA was isolated from dermal tissues and DLN of NC/Nga mice and used for RT-PCR analysis. TNF-α mRNA levels of GSMT-treated animal in terms of RQ values were 0.458 which was lower than that of the control (RQ=1) (Fig 5). IL-6 mRNA levels in the dermal tissue of GSMT-treated animal in terms of RQ values were 0.744, which was lower than the RQ value in the control group. IL-4 mRNA levels of GSMT-treated animal were 0.784. IL-13 mRNA levels of GSMT-treated animal were 0.156, showing much decrease compared with the control group. IFN-γ mRNA levels of GSMT-treated animal in terms of RQ values were 0.545, a half level to the control group.

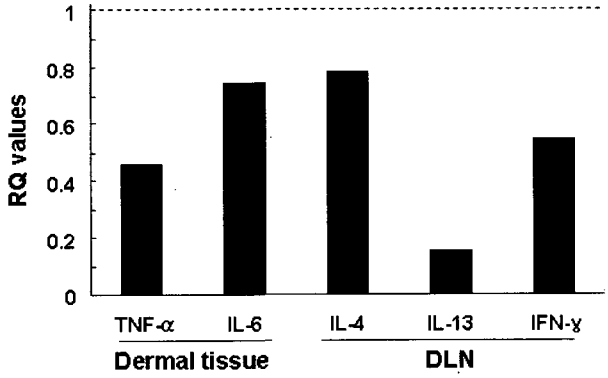

Fig. 5. Quantitative real-time PCR of dermal tissue and DLN mRNA. The amount of SYBR Green was measured at the end of each cycle. The cycle number at which the emission intensity of the sample rises above the baseline is referred as to the RQ (relative quantitative) and is proportional to the target concentration. Real time PCR was performed in duplicate and analyzed by a Applied Biosystems 7500 Fast Real-Time PCR system.

6. Histological examination

H & E staining of ear, skin, and DLN tissues showed increased edema in the epidermis and dermis and increased infiltration of leukocytes in the control animals. In individual tissues of GSMT-treated animals, epidermal edema and leukocyte infiltration was much decreased, indicating a large improvement of edema (Fig. 6).

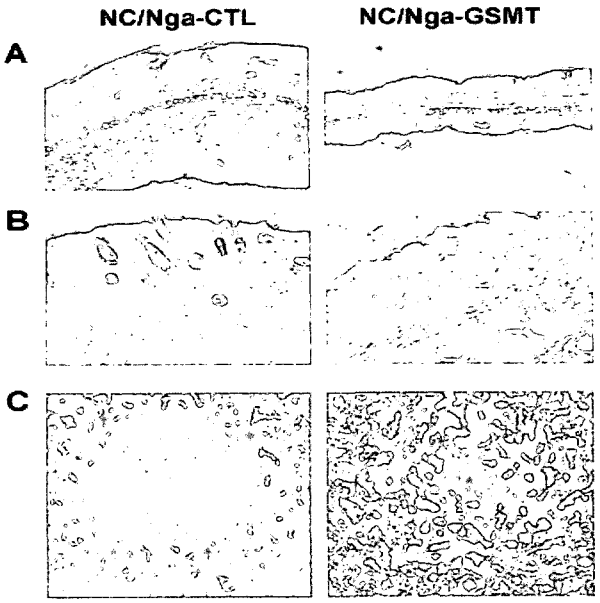

Fig. 6. Histologic features of several tissues of NC/Nga mice. NC/Nga mice were administered with GSMT (15 mg/25g, B) for 8 weeks. Mouse ear (A), skin (B), and DLN (C) biopsies were stained with H&E. Levels of infiltration of the inflammatory lymphocytes cells (ILC) and plasma cells (PC), marked by arrows, were mostly disappeared in GSMT-treated group. The image was taken with 100x magnification under bright field microscopy.

Discussion

One of the prominent features of oriental medicinal therapy over western medication is that it considers patient's status or four constitution, which is termed 'Sasang' in Korean. For instance, Gamisamul-tang (GSMT) used in the present study is one of the prescriptions to supplement the blood and 'yin' spirit to 'Soyang' person. GSMT along with a few other prescriptions are known to discharge 'heat' in the blood and body, thereby relieving allergic reaction known to be negatively deteriorating atopic responses of the skin. Pathogenesis of many immune diseases (i.e., hypersensitivity and autoimmune diseases) is complicated particularly because both genetic and environmental factors appear to be involved. These factors are taken more seriously for the diagnosis in the oriental medicine than western medicine.

In the present study, GSMT was investigated in relation to AD development using NC/Nga AD mouse model. NC/Nga strain was originated from Japanese fancy mice and established as an inbred strain[7]. This strain has been reported to have features such as a high susceptibility to X-irradiation and to anaphylactic shock induced by ovalbumin. NC/Nga mice develop AD-like eczematous skin lesions when kept in an air-uncontrolled conventional room[15-17]. NC/Nga mice also develop hypersensitivity responses by a repeated application of haptens such as 2,4,6-trinitrochlorobenzen (TNCB), and this hypersensitivity is associated with epidermal hyperplasia,

accumulation of large numbers of mast cells and $CD4^+$ T cells beneath the epidermis, and elevated serum levels of antigen-specific IgE[18,19].

In the present study, AD was clearly developed in DNCB-treated NC/Nga mice. Major changes included increased activation of immune cells such as $CD4^+$ and $CD8^+$ T cells and NK cells and increased production of IL-4, IL-5, and IL-13 cytokines, as well as IgE, IgG and IgM antibodies. These responses correlated well with the development of AD symptoms and histological changes in the skin and DLN as well as increased clinical severity scores. Increased synthesis of mRNA encoding several kind of cytokine proteins, as determined by RT-PCR, indicates up-regulation of inflammatory cytokines at the gene expression level. It was previously shown that IL-4 and IL-5 were produced by mast cells and $CD4^+$ T cells in lesional skin, while IFN-γ was also produced by a small number of these cells when NC/Nga mice were induced for AD[20]. Defective production of IFN-γ by T cells in the NC/Nga mice seemed to be associated with IgE hyperproduction. This notion is consistent with the present data representing decreased production of IFN-γ and increased IL-4 and IL-5 in activated NC/Nga mice.

Administration of GSMT into NC/Nga mice generated AD-related changes in NC/Nga mice. GSMT down-regulated levels of several inflammatory cytokines known to be induced during hypersensitivity immune reaction in AD tissues. Similarly, increased levels of $CD4^+$ and $CD8^+$ T lymphocytes in AD-induced lymph node returned to normal levels by GSMT treatment. IL-4, IL-5, and IL-13 in the serum were reduced to levels close to those in the normal animals. The present data further suggest that several cytokines such as IL-4, IL-6, IL-13, TNF-α and IFN-γ were down-regulated at the gene expression levels. These cytokines are the major intial trigger for a systemic expansion of Th2 cell activity, again leading to releases of the same and other inflammatory cytokines which cause eosinophilia, increase IgE, and increase the growth and development of mast cells[21,22].

Thus, the present data suggest that the treatment of GSMT regulated levels of several chemical mediators related to the progression of AD. It is expected that GSMT may be effective for the cure of atopy dermatitis by regulating the complementation of blood and 'yin', removal of chilliness and fever relief and detoxification. Possible molecular mechanisms linking clinical efficacy of GSMT to regulation of molecular components related to hypersensitivity immune reactions are not known at this moment, but it is a possibility that multiple factors in the herbal drugs could make interaction to individual immune cells.

Another important finding from the present study is the observation of the recovery from AD at histological level by GSMT. Clinically, AD is well observed under the category of eczematous dermatitis, and histologically spongiotic dermatitis in the epidermis, indicating the accumulation of edema fluid within the epidermis, characterizes atopic dermatitis[23,24]. Consistent with previous reports, increased epidermal edema and increased infiltration of immune cells were observed in NC/Nga mice. Then, these changes indicating clinical features of AD were largely eliminated by GSMT administration. Tissue morphology and histological examination showed the recovery similar to normal tissue.

The present data therefore strongly suggest that oriental medicinal prescription can effectively regulate AD symptoms in an experimental animals as similarly implicated in the clinical performance. It should be noted that there have been several studies raising the possible application of alternative medicine on AD therapy. For instance, it has been shown that persimmon leaf extract and astragalin were effective in inhibiting the development of dermatitis[25]. The persimmon leaf contains antiallergic substances inhibiting histamine release by human basophilic cell. Oral intake of this extract into NC/Nga mice significantly reduced not only infiltration of inflammatory cells, especially degranulated mast cells, thickening of the epidermis, and prominent hyperkeratosis, but also down-regulated the capacity of spleen T cells to produce both IL-4 and IL-13, but not IFN-γ. Similar physiological effects such as skin symptoms and transepidermal water loss of persimmon leaf extract were reported[26]. Also, administration of royal jelly suppressed the development of AD-like skin lesions in NC/Nga mice[27]. Besides the potential effects of natural herbal drugs mentioned above, steroid drugs such as dehydro-epiandosterone and 2,3,7,8-tetrachlorodibenzo-p-dioxin were also effective in attenuating atopic dermatitis responses in NC/Nga mouse. Together, these studies suggest the possibility that atopic dermatitis can be regulated by appropriate natural drugs as well as chemical drugs. In this aspect, the present findings on the attenuating effects of GSMT treatment on AD provide a broad insight into the use of natural herbal products for AD therapy.

The present findings along with pervious reports as mentioned above have been examined in the experimental animal model, and thus further investigation of whether the effects could be reproducible in the human remains to be explored. It was reported that about 50% of NC/Nga mice displayed the incidence of AD-like lesions although histopathological characteristics of atopic dermatitis in

NC/Nga mouse model are very similar as occurs in human atopy[19,28]. This is important because there are certainly much more complexities and individual variations reflecting genetic predispositions and environmental influences in humans above AD-induced mouse. In conclusion, the present finding indicates possible way of identifying molecular and cellular factors that might be critical for AD progression and regulated by oriental medicinal drugs.

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