

ORIGINAL ARTICLE

Daily intake of Jeju groundwater improves the skin condition of the model mouse for human atopic dermatitis

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ABSTRACT

Drinking water is an important nutrient for human health. The mineral ingredients included in drinking water may affect the physical condition of people. Various kinds of natural water are in circulation as bottled water in developed countries; however, its influence on clinical conditions of patients with certain diseases has not been fully evaluated. In this study, effects of the natural groundwater from Jeju Island on clinical symptoms and skin barrier function in atopic dermatitis (AD) were evaluated. NC/Tnd mice, a model for human AD, with moderate to severe dermatitis were used. Mice were given different natural groundwater or tap water for 8 weeks from 4 weeks of age. Clinical skin severity scores were recorded every week. Scratching analysis and measurement of transepidermal water loss were performed every other week. The pathological condition of the dorsal skin was evaluated histologically. Real-time polymerase chain reaction analysis was performed for cytokine expression in the affected skin. The epidermal hyperplasia and allergic inflammation were reduced in atopic mice supplied with Jeju groundwater when compared to those supplied with tap water or other kinds of natural groundwater. The increase in scratching behavior with the aggravation of clinical severity of dermatitis was favorably controlled. Moreover, transepidermal water loss that reflects skin barrier function was recovered. The early inflammation and hypersensitivity in the atopic skin was alleviated in mice supplied with Jeju groundwater, suggesting its profitable potential on the daily care of patients with skin troubles including AD.

Key words: animal model, atopic dermatitis, itch, natural groundwater, transepidermal water loss.

INTRODUCTION

Jeju Island is located at the Korean southernmost tip, and the warm Tsushima current flows around the island. The climate of Jeju Island is the warmest in the Korean Peninsula with much rainfall. Jeju Island appeared after a large volcanic eruption in the sea approximately 700 000–1200 000 years ago. Mount Halla, which is the highest shield volcano in South Korea, rises from the center of Jeju Island, and provides the geographical features with parasitic cones peculiar to Jeju Island which are called “oreum”. The volcano bedrock layer that has been sterilized by the high temperatures of volcanic eruptions, serves as a natural filter for filtration of groundwater, and provides soft water which has low mineral contents. Therefore, groundwater in Jeju Island, which is drawn up from a depth of 420 m, is one of the greatest sources of Asian natural water to drink. In recent years, many consumers who drink the natural groundwater of Jeju Island every day state that itch and xerosis of the

skin have been relieved; however, scientific evaluation has not been carried out yet.

Atopic dermatitis (AD) is a chronic and relapsing skin disorder with allergic immune responses resulting in the hyperproduction of immunoglobulin (Ig)E.¹ Most of the patients have genetic cofactors promoting manifestation and/or a family history of allergic disorders, including anamnesis and/or bronchial asthma, allergic rhinitis and conjunctivitis. Abnormality in the immune system and skin barrier function gives rise to serious problems in patients with AD.² Contact or invasion of environmental antigens such as house dust mites exacerbates clinical symptoms. Itch is one of the most serious clinical symptoms of AD, and repeated itching–scratching episodes may be greatly involved in the acceleration and severity of dermatitis.^{1,3,4} Epidermal hyperplasia, as well as infiltration of inflammatory cells, is obvious in the affected skin of patients with AD.³ Thymic stromal lymphopoietin (TSLP) is released from growing keratinocytes by stimulation of antigens.⁵ TSLP induces early

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progression of allergic responses by activating dendritic cells.^{5,6} Activated dendritic cells produce various inflammatory cytokines that are responsible for development of AD.^{5,6} Hyperproduction of nerve growth factor (NGF) from proliferative keratinocytes and fibroblasts in the skin promotes sprouting of sensory neurons resulting in itch sensation in the skin lesions.^{7,8} Regulatory T cells (Treg) are known to be critically involved in the regulation of allergic immune responses.⁹ Defect in the function of Treg has been suggested as one of the causes of immune-mediated diseases. However, the involvement of Treg in the onset and development of AD has been unclear. Because the background is multifactorial and the underlying predisposition are involved, the pathogenesis of AD is quite complex.

NC/Tnd mice, an inbred strain originated from Japanese fancy mice, spontaneously develop itchy dermatitis quite similar to AD in an air-unregulated conventional circumstance from 6–8 weeks of age.^{10–16} NC/Tnd mice have been used for investigation in pathogenesis of AD and for development of new therapies for AD all over the world. In the current study, we examined whether daily supplementation of Jeju groundwater may have beneficial effects on clinical symptoms and skin barrier function with a model for human AD, NC/Tnd mice.

METHODS

Mice

In a previous study, we used conventional NC/Tnd mice, a model for human AD. Specific pathogen-free NC/Tnd mice were transferred and maintained in an air-controlled condition from 4 weeks of age before the onset of dermatitis. Mice were accustomed for 1 week in conventional conditions prior to starting the supplementation. Mice were kept in a clear acrylic cage and allowed free access to standard chow and water. The temperature and humidity of the animal room were $22 \pm 4^\circ\text{C}$ and $40 \pm 15\%$, respectively. The animal room was maintained on a 12:12-h light : dark cycle. All experiments with animals complied with the standards specified in the guidelines of the University Animal Care and Use Committee in Tokyo University of Agriculture and Technology.

Mice ($n = 10/\text{group}$) were allowed free access to Jeju groundwater (Sam Da Soo), two different kinds of commercially available natural water (A and B), or tap water of Tokyo city before delivery till 12 weeks of age. Water was replaced twice a week. The amount of drinking water consumed by individual mice in each group was not statistically significantly different.

Endotoxin and minerals in water

The concentration of endotoxin was determined by a limulus test. Flame atomic absorption spectrophotometry was performed for detection of iron, manganese, copper, zinc, sodium, potassium, calcium, magnesium and molybdenum. Inductively-coupled plasma-optical emission spectrometry was used for analysis of total chromium, vanadium and cobalt. Selenium was measured by hydride generation atomic absorption spectrometry. Iodide, total sulfur and total phosphorus were

assayed by iodometric titration, gravimetric and peroxodisulfate digestion methods, respectively. Colorimetric analysis with molybdenum yellow was applied for silica, and that with mercury (II) thiocyanate was used for chloride. Water hardness was calculated according to the American method: $\text{Ca}^{++} (\text{mg/L}) \times 2.5 + \text{Mg}^{++} (\text{mg/L}) \times 4$ (p.p.m.). All analysis for endotoxin and minerals in water was performed by Kurita Analysis Service (Kanagawa, Japan). Endotoxin and mineral contents of the water are listed in Table 1.

Clinical skin severity scores

According to the criteria of Matsuda *et al.*,¹² the total clinical skin severity score graded 0–3 for AD-like lesions was defined as the sum of the individual scores. Grading criteria was applied for each of the five signs (itch, erythema/hemorrhage, edema, excoriation/erosion, scaling/dryness) and symptoms. Clinical skin severity scores were recorded every week by researchers who were blinded to the water supplied to each group.

Analysis of scratching behavior

Because itch is one of the most unbearable symptoms in AD, scratching behavior of individual mice was recorded and analyzed by a quantification system for scratching behavior of mice (SCLABA-Real; Noveltec, Kobe, Japan)^{6,17–21} at 4-week intervals. The SCLABA-Real system measures the frequency and duration of scratching episodes in fixed periods for each mouse. Briefly, without marking, mice were put into the acrylic boxes and allowed to adapt for 30 min before measurement commenced. The behavior of four mice was simultaneously recorded by a high-speed digital camera from the top of the exclusive cage by near-infrared light. SCLABA-Real software accurately detects the algorithm that is specific for scratching behavior in mice and records data in real time. SCLABA-Real calculates “frame-to-frame difference”, that is a mouse’s migration area per frame by capturing animal behavior at a rate of 240 frames/s using the high-speed camera containing an image processing system. This whole new system allows us to quantify the number and duration of scratching behavior automatically by time-course analysis of “frame-to-frame difference” and evaluate the degree of itching from many directions.

Transepidermal water loss (TEWL)

Stratum corneum (SC) hydration is important for its cosmetic properties and barrier function. TEWL is one of key indexes used for SC characterization, which reflects barrier function of the skin. In patients with AD, TEWL is increased as the disease advances.^{16,22} TEWL of each mouse was measured every 4 weeks during the experiment by using a Multi probe adopter (CK Electronic, Cologne, Germany). At the time of measurement, mice were allowed to adapt to the laboratory conditions where temperature and humidity were set at 23°C and 40%, respectively, for 30 min prior to the measurement. Measurement for each mouse was performed three times and the average of three values was estimated as the data.

Table 1. Endotoxin, mineral ingredients, pH, and hardness of water used in the current study

Analyzed items		Analyzed water			Jeju groundwater
		Tap water	A water	B water	
Endotoxin (EU/ml)		0.095	1.300	0.243	<0.025
Mineral ions (mg/L)	Chloride	10	7	5	7
	Silicate	39	15	26	28
	Phosphate	0.04	<0.03	0.03	0.09
	Sodium	12.70	6.55	7.22	5.67
	Potassium	2.76	1.14	3.01	2.33
	Calcium	39.30	72.40	6.50	1.80
	Magnesium	7.00	25.40	1.39	1.39
	Vanadium	<0.001	<0.001	0.001	0.008
pH		7.0	7.2	7.0	7.8
Water hardness		126.3	282.6	21.8	10.1

Plasma total IgE

Plasma total IgE concentrations were measured by an enzyme-linked immunoassay as described previously.^{12,13} Blood samples were collected from the abdominal aorta of each NC/Tnd mouse in the four groups at the end of experiments. The collected blood samples were stored at room temperature for 30 min to facilitate aggregation of fibrin. Serum samples were collected after centrifugation of blood samples at 800 *g* for 15 min to precipitate aggregated fibrin and blood cells. Plasma samples were stored at -20°C until use. Absorbance was measured by using ImmunoMini NJ-2300 (Biotec, Tokyo, Japan) with a 450-nm filter.

Histological examination

Histological analysis of the affected skin lesion was performed as previously described.⁶ Briefly, at the end of the experiment, the back skin of each mouse was shaved with a hair clipper, cut into small pieces, and fixed by immersion in 10% neutral buffered formalin for 24 h at room temperature. Tissue samples were embedded in paraffin. The paraffin blocks were sectioned using a 6- μm -thick microtome. Paraffin sections were mounted on slides and air-dried overnight. The deparaffinized sections were stained with hematoxylin–eosin, and evaluated by microscopic observation. Mast cells were identified in tissue sections after staining with toluidine blue (pH 4.1). To identify eosinophils in the skin, skin sections were stained with Congo red. Numbers of mast cells and eosinophils were counted ($3 \times$ high-power fields [HPF] at random) under a microscope and the average was taken as a value of the sample.

To detect CD4⁺ T cells, frozen sections were incubated with affinity-purified anti-CD4 antibody (BD Pharmingen, San Diego, CA, USA).⁶ Each preparation was then incubated with peroxidase-conjugated streptavidin and stained in a substrate solution containing hydrogen peroxide and 3-amino-9-ethylcarbazole (Dako, Glostrup, Denmark). The number of CD4⁺ T cells was counted under a microscope at a magnification of $\times 400$, and were expressed as the total number of cells in five fields.

All the slides were examined under a light microscope (Nikon ECLIPSE E600; Nikon, Tokyo, Japan) and images were

captured using a DXM1200F digital camera with Nikon ACT-1 software.

RNA isolation and real-time reverse transcription polymerase chain reaction (RT–PCR)

The dorsal skin and the spleen samples were isolated from each NC/Tnd mouse at the end of the experiment as described previously.^{20,21} A 6-mm diameter of the skin and one-third of the spleen were snap frozen and homogenized with Multi-beads shocker (Yasui Kikai, Osaka, Japan). Total RNA was extracted from each sample with ISOGEN (Nippongene, Tokyo, Japan). To detect gene expression of various inflammatory cytokines, real-time RT–PCR analysis was performed as previously described.^{20,23} Reaction mixtures were amplified with a SYBR Green PCR Master Mix (Applied Biosystems, Tokyo, Japan) in the presence of 0.2 μM of the sense and antisense primers as follows: interleukin (IL)-4, forward 5'-TCTCGAATGTACCAGGAGCCATATC-3', reverse 5'-AGCACCTTGGAAGCCCTACAGA-3'; IL-5, forward 5'-AGCACAGTGGTGAAAGAGACCTT-3', reverse 5'-TCCAATGCATAGCTGGTGATT-3'; interferon (IFN)- γ , forward 5'-CGGCACAGTCATTGAAAGCCTA-3', reverse 5'-GTTGCTGATGGCTGATTGTC-3'; IL-10, forward 5'-GACCAGCTGGAC AACATACTGCTAA-3', reverse 5'-GATAAGGCTTGGCAACCCAA GTAA-3'; transforming growth factor (TGF)- β 1, forward 5'-GTGTGGAGCAACATGTGGAACCTCTA-3', reverse 5'-TTGGTTCA GCCACTGCCGTA-3'; TSLP, forward 5'-CGAGCAAATCGAGG ACTGTGAG-3', reverse 5'-GCAGTGGTCATTGAGGGCTTC-3'; NGF, forward 5'-TGCCAAGGACGCAGCTTTC-3', reverse 5'-TG AAGTTTAGTCCAGTGGGCTTCAG-3'; Foxp3, forward 5'-AGTG CCTGTGTCCTCAATGGTC-3', reverse 5'-AGGGCCAGCATAGG TGCAAG-3'; and β -actin, forward 5'-TGACAGGATGCAGAA GAGA-3', reverse 5'-GCTGGAA GGTGGACAGTGAG-3'.

The thermal cycling programs was set as follows: stage 1, 95°C for 30 s; stage 2, 40 cycles of 95°C for 5 s and 60°C for 31 s; and stage 3, 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. Fluorescence intensity was measured in real time during extension steps for a SYBR Green assay by using the ABI Prism 7000 Sequence Detector (Applied Biosystems). Relative mRNA expression of the target genes was determined by the

$2^{-\Delta\Delta CT}$ method. Each value was presented as fold change of mRNA expression by calculating the average mRNA expression level of mice supplied tap water as 1.

Statistical analysis

Clinical skin severity scores, scratching analysis and TEWL values were compared among the four groups by ANOVA and non-parametric Dunnett's multiple comparison by using JMP ver. 9.0.0 (SAS Institute, Tokyo, Japan). Data from histological grading and inflammatory cell counting were also compared among the four groups by ANOVA and non-parametric Dunnett's multiple comparison using JMP ver. 9.0.0 (SAS Institute). All data represent the mean $\pm$ standard error of the mean (SEM).

Serum IgE concentrations were compared among the four groups by ANOVA and Tukey–Kramer test as post-hoc analysis. Statistical analysis was performed using Ekuseru-Toukei 2008 software (Social Survey Research Information, Tokyo, Japan). $P < 0.05$ was considered statistically significant. All data represent the mean $\pm$ SEM.

Inflammatory cell numbers in histological analysis were compared with data from the tap water group by Student's *t*-test using JMP ver. 9.0.0 (SAS Institute).

Relative mRNA expression levels of each gene were compared among the four groups by ANOVA and Tukey–Kramer test as post-hoc analysis. Statistical analysis was performed using Ekuseru-Toukei 2008 software (Social Survey Research Information). $P < 0.05$ was considered statistically significant. All data represent the mean $\pm$ SEM.

RESULTS

Changes in clinical skin severity scores

At the beginning of the experiment, clinical skin severity scores varied 6.2–7.4 in each group, and there was no statistical difference between groups (Fig. 1). Clinical skin conditions in mice treated with tap water were aggravated gradually over 8 weeks. Clinical scores of mice treated with A water were also increased as compared with those at the beginning (6.2 ± 1.4 to 9.6 ± 1.4), indicating that dermatitis became more severe at the end of the experiment in these two groups (Fig. 1). Dermatitis in mice receiving B water was not improved (7.4 ± 0.7 to 8.4 ± 0.6), but inactivated the serious increase during the experimental period (Fig. 1). On the other hand, exacerbation of clinical symptoms in mice receiving Jeju water was suppressed (6.2 ± 1.3 to 6.6 ± 0.9) (Fig. 1). Clinical scores in mice receiving Jeju water lowered significantly from 5 weeks after the experiment started.

Scratching analysis

Scratching episodes over a fixed period in mice supplied with tap water increased week after week (Fig. 2). The increase in scratching frequency of mice treated with A water and B water was not remarkable, but was not relieved at all. On the other hand, scratching frequency in mice receiving Jeju water was reduced significantly over 8 weeks (Fig. 2a). In the tap water group, scratching duration at each time point was significantly increased (Fig. 2b). The increase in scratching duration of mice

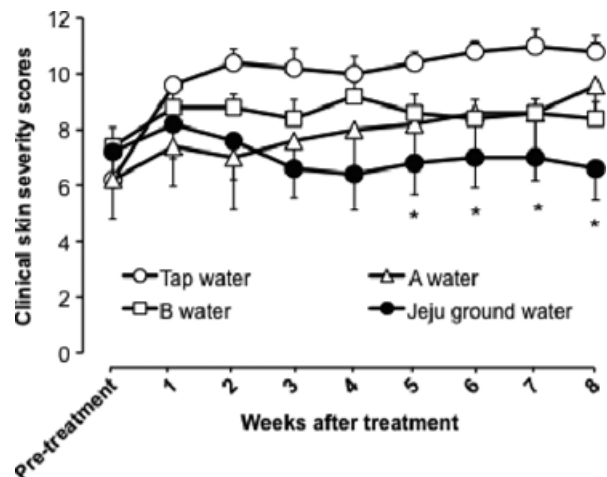


Figure 1. Changes in clinical skin severity scores. Each value represents means $\pm$ standard error of the mean of 10 mice in each group. * $P < 0.05$, when compared with control group given tap water.

treated with A water and B water was not obvious, but was not reduced at all. On the other hand, as well as the data from scratching frequency analysis, scratching duration in mice treated with Jeju water was decreased over 8 weeks with statistical significance (Fig. 2b).

TEWL measurements

The normal values of TEWL in laboratory mice are less than 10 g/h per m^2 . As inflammatory dermatitis progresses, TEWL increases, which indicates destruction of the skin barrier. Particularly, scratching behavior induces breakdown of the skin barrier and accelerates the increase of TEWL. In mice receiving A water and tap water, TEWL was raised during the experimental period (Fig. 3). Particularly, the increase in TEWL in the tap water group was obvious. On the other hand, TEWL measured in mice supplied with B water was unchanged (Fig. 3). TEWL in mice treated with Jeju water was significantly suppressed when compared with that in mice treated with tap water after 8 weeks of treatment (Fig. 3).

Plasma total IgE measurement

To determine the effects of drinking water on immune responses in NC/Tnd mice, we measured plasma total IgE concentrations. IgE concentrations varied 3.12–3.50 g/mL in each group. There was no significant difference in plasma total IgE among the four groups after the experiment (data not shown).

Histological examination

Hematoxylin–eosin staining revealed remarkable epidermal hyperplasia and infiltration of inflammatory cells in dermis in the dorsal skin of mice receiving tap water (Fig. 4a). Histological characteristics of the dorsal skin from mice in groups receiving A or B water were better than those of mice supplied with tap water to some extent, but significant improvement was not evident (Fig. 4a). Pathohistological features of mice

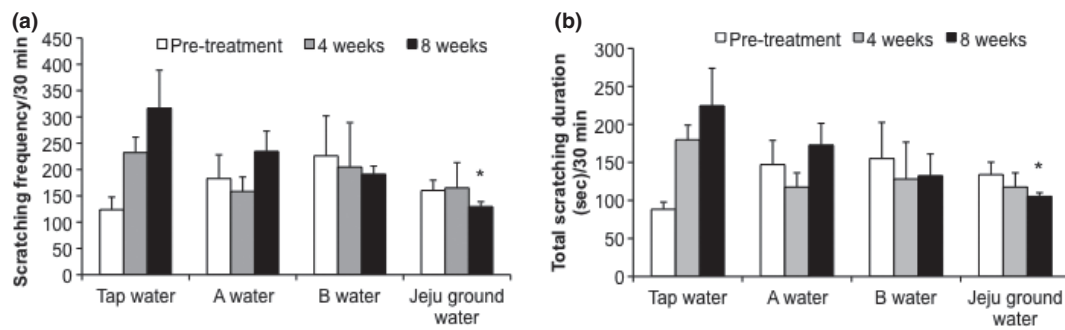


Figure 2. Changes in (a) scratching frequency and (b) total duration (s). Each value represents means $\pm$ standard error of the mean of 10 mice in each group. * $P < 0.05$, when compared with control group supplied tap water for 8 weeks.

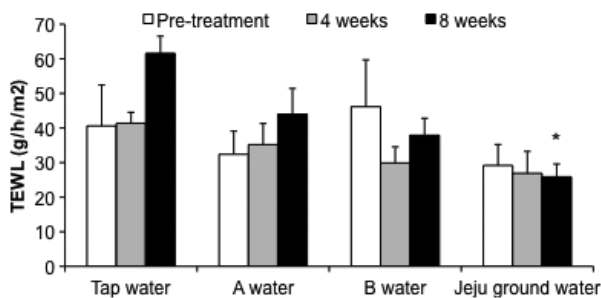


Figure 3. Changes in transepidermal water loss (TEWL). Each value represents means $\pm$ standard error of the mean of 10 mice in each group. * $P < 0.05$, when compared with control group supplied tap water for 8 weeks.

supplied with Jeju water seemed to be improved in terms of hyperkeratosis, acanthosis, spongiosis and dermal inflammation (Fig. 4a).

Mast cell numbers in the dorsal skin were approximately 80 cells/HPF ($\times 400$) in mice of the tap water group (Fig. 4b). In mice treated with A or B water, there were a similar number of mast cells in the dorsal skin. On the other hand, the number of mast cells in mice supplied Jeju water tended to decrease, but there was no statistical significance. Eosinophil numbers in the dorsal skin were approximately 10 cells/HPF in mice of the tap water group (Fig. 4b). In mice of the A or B water group, there was a similar number of eosinophils in the dorsal skin. On the other hand, eosinophil numbers were significantly decreased in mice of the Jeju water group. CD4⁺ cell numbers were significantly reduced in mice treated with Jeju groundwater or B water, when compared to those in mice receiving tap water (Fig. 4b). Dendritic cells were detected by immunohistochemistry; however, no significant difference among the four groups was recognized (data not shown).

Cytokines and Foxp3 analysis

To determine the effects of drinking water on immune responses in the NC/Tnd mice, we measured a variety of cytokine mRNA expressions in the skin and spleen. TSLP expression in the skin was not different among the four groups;

however, that in mice supplied with A water tended to be high (Fig. 5). NGF expression in the skin of mice receiving A water was significantly higher than that in the other groups (Fig. 5). In the spleen, significant difference of IL-4, IL-5, IFN- γ , IL-10 and TGF- β 1 mRNA expression was not recognized among the four groups (data not shown).

To identify the mechanism that alleviated the severity of dermatitis, we attempted to check Treg after supplementation of each water. After 8 weeks of treatment, Foxp3 expression in the spleen and the lymph nodes was examined. In mice supplemented with Jeju groundwater, the expression level of Foxp3 was significantly upregulated when compared to that in mice supplied with tap water, A water or B water (Fig. 6).

DISCUSSION

In the current study, the importance of drinking water in better control of AD was first demonstrated. Various opinions have been expressed on the effect of drinking water on skin conditions of AD. However, evidence based on reliable scientific research was lacking. For the first time, we herein provide new insight into the beneficial role of drinking water in regulation of AD using a model for human AD, NC/Tnd mice.

A water, which is natural groundwater in Europe, has higher concentrations of both calcium and magnesium, which is so-called "hard" water. B water, which is Japanese natural groundwater, is "soft" water with lower concentrations of both calcium and magnesium. Jeju natural groundwater has much lower levels of those two minerals, which is called "very soft" water. These three kinds of natural groundwater are commercially available as bottled water that can be purchased at supermarkets. Aggravation of clinical skin conditions in mice receiving Jeju groundwater as drinking water was significantly suppressed, indicating that the water may have a certain favorable effect in relief of AD. Analysis of scratching frequency and total scratching duration supported the result obtained in Figure 1. In mice supplied with tap water, scratching behavior increased weekly, as well as the quality of itch sensation. On the other hand, scratching behavior of mice treated with Jeju groundwater was not exacerbated, demonstrating that itch sensation in mice treated with the water was, at least in part, relieved.

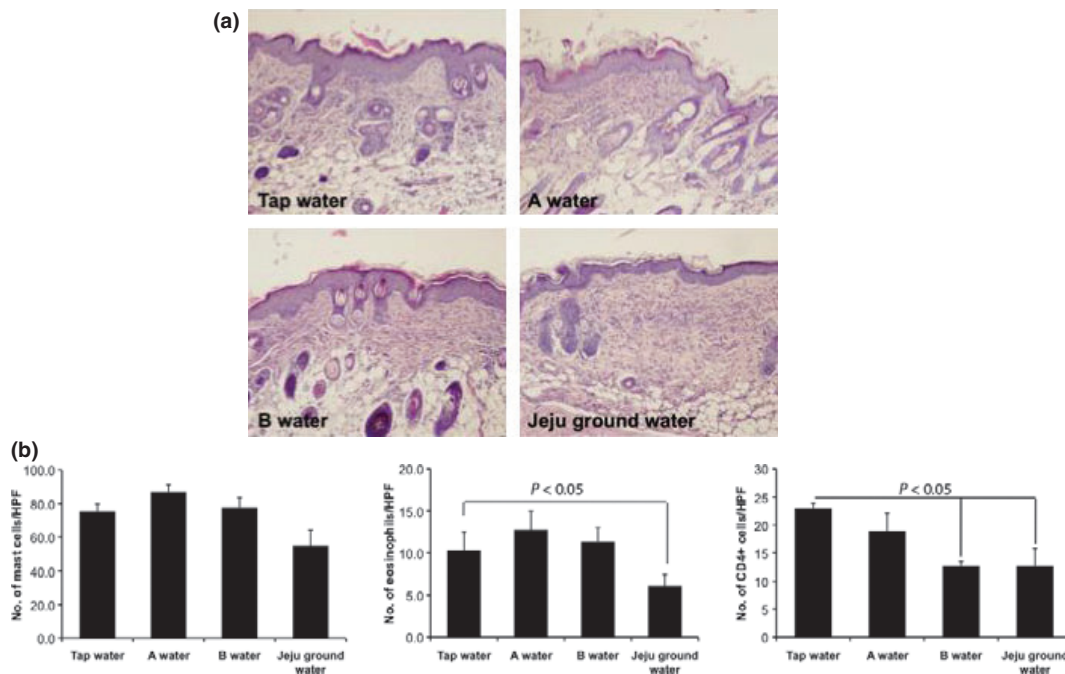


Figure 4. (a) Pathological features and (b) numbers of inflammatory cells of the dorsal skin of mice in each group (hematoxylin-eosin, original magnification $\times 40$). Each value represents means $\pm$ standard error of the mean of five mice in each group. $P < 0.05$, when compared with control group supplied tap water. HPF, high-power field.

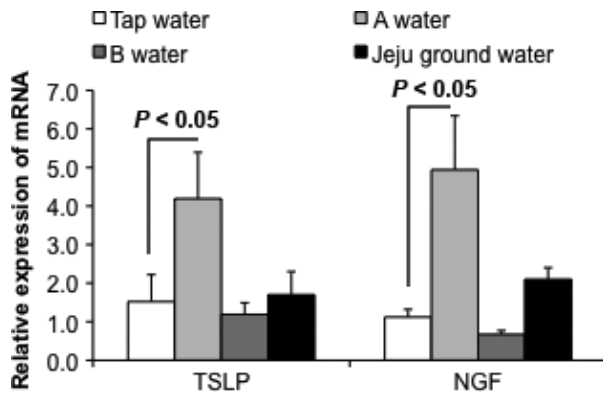


Figure 5. Cytokine mRNA expression in the dorsal skin of NC/Tnd mice in each group. Each value is presented as fold change of mRNA expression by calculating the average mRNA expression level of the tap water group as 1. Data represent means $\pm$ standard error of the mean of five mice in each group. $P < 0.05$, when compared with the mice supplied tap water. NGF, nerve growth factor; TSLP, thymic stromal lymphopoietin.

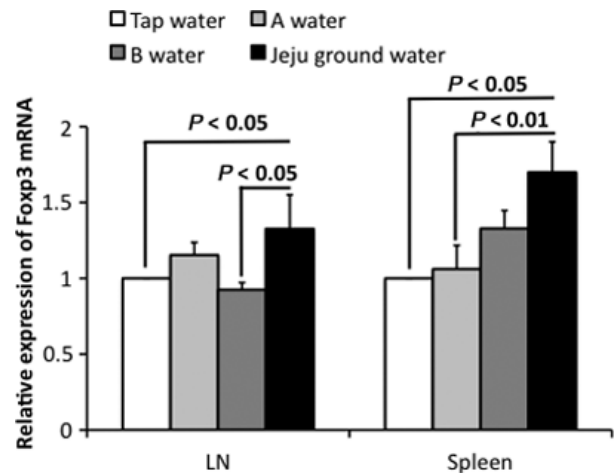


Figure 6. Foxp3 mRNA expression in the spleen and lymph nodes of NC/Tnd mice in each group. Each value is presented as fold change of mRNA expression by calculating the average mRNA expression level of the tap water group as 1. Data represent means $\pm$ standard error of the mean of five mice in each group. $P < 0.05$, when compared with the mice supplied tap water. LN, lymph node.

Although levels of plasma total IgE were not reduced, data from TEWL measurement revealed the surprising effect of Jeju groundwater on barrier maintenance of the skin even in atopic circumstances. The results obtained from TEWL analysis were supported by pathological examinations of the affected dorsal

skin. Pathological features and numbers of allergic inflammatory cells in mice supplied with Jeju groundwater were well-controlled, suggesting that the water had some beneficial effects on

turnover of the skin barrier and relief of allergic inflammation. A variety of calcium-binding proteins with calcium-binding hands have been reported to cross-sensitize cell-bound IgE with other environmental antigens in allergic individuals.^{24,25} Calcium-binding allergens are known to upregulate IgE-mediated allergic reaction.^{24,25} The recognition of calcium-binding proteins with IgE depends on calcium concentrations.^{24,25} Although intake of water with a high concentration of calcium may facilitate the activity of calcium-binding proteins, further examination must take place to elucidate whether calcium concentration in the skin would be increased by uptake of hard water.

Orally supplied water may change systemic immune responses through a gut-associated mechanism. It is reported that beneficial effects of probiotics on AD was partially induced through Treg function.^{26,27} Although involvement of the gut environment in severity of dermatitis has been unclear, we found that Foxp3 expression was upregulated systemically in mice supplemented with Jeju groundwater. These results raise the possibility that induced Foxp3⁺ Treg may relieve the severity of dermatitis, though further investigation regarding effects of Jeju groundwater on the gut microorganism must take place. Interestingly, expression levels of TSLP and NGF were relatively high in mice supplied with A water. Hardness of A water is quite high; on the other hand, that of Jeju groundwater and B water is very or rather low. Moreover, endotoxin content was the highest in A water among the four groups. Although the association of nutrients and systemic inflammation has been suggested,²⁸ scientific examination of this important issue has not been completed. Further experiments must take place; however, soft water without endotoxin and calcium ions has a certain regulative effect on the early allergic responses induced by TSLP and NGF.

Taken together, the groundwater from Jeju Island has extremely profitable potential as drinking water in the daily care of AD through the regulation of skin barrier function and itch sensation, indicating quite helpful effects on maintenance of skin conditions without unfavorable side-effects.

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