

Glucosamine Improved Atopic Dermatitis-like Skin Lesions in NC/Nga Mice by Inhibition of Th2 Cell Development

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Abstract

Dysregulated Th subset responses, characterized by Th2-dominant allergic inflammation, are thought to be central to the pathogenesis of atopic dermatitis (AD). Glucosamine has been shown to have immunosuppressive properties, but its effect on AD has not been examined. In this study, the immunoregulatory effects of glucosamine, using dermatophagoides farinae (Df)-induced AD-like skin lesions in NC/Nga mice, were investigated. The clinical scores were reduced significantly by the treatment with glucosamine at 10 and 20 mg/day. Histological analysis of the skin also revealed that treatment of glucosamine at 10 and 20 mg/day significantly reduced the inflammatory cellular infiltrate, including mast cells and eosinophils. The levels of serum IgE and Th2 cytokines in spleen cells were reduced, whereas no significant change was detected in IFN- γ , a Th1 cytokine. To determine the mechanism associated with inhibition of the Th2 immune response, the effects of glucosamine on the selective differentiation pathway of the Th subset *in vitro* was examined in NC/Nga mice. The results showed that glucosamine suppressed the differentiation of naïve CD4⁺ T cells to Th2 cells *in vitro*. On the basis of *in vivo* and *in vitro* results of the NC/Nga mice, the immunobiological effects of glucosamine on peripheral blood mononuclear cells from patients with AD were examined. The production of Th2 cytokines, such as IL-4 and IL-5, was significantly decreased after *in vitro* administration of glucosamine, which suggest that glucosamine might be a useful immunomodulatory agent for the treatment of human AD.

Introduction

Atopic dermatitis (AD) is an inflammatory, chronically relapsing, non-contagious pruritic skin disorder [1, 2]. AD is caused by the complex interaction of genetic, pharmacological, environmental and psychological factors that result in immune system dysregulation [3, 4]. Dysregulated T-helper (Th)1 and Th2 responses characterized by Th2-dominant allergic inflammation are thought to be central to the pathology of diseases such as AD and asthma [5, 6]. Particularly, Th2 immune responses mediated by interleukin (IL)-4, IL-5 and IL-13 are key to the pathogenesis of atopic disorders [7]. The presence of blood eosinophilia and increased serum IgE levels in the majority of patients with AD supports Th2 dominance in AD [8].

The CD4⁺ Th2 cells involved in AD are differentiated from naïve CD4⁺ T cells. For the differentiation, T cell receptor (TCR) and cytokine-mediated signalling upregulate various transcription factors [9, 10]. GATA-3 is a transcription factor that plays a critical role in Th2 differentiation [11], whereas the signal transducer and activator 4 (STAT4) and T-box expressed in T cells (T-bet) are directly involved in the formation of activating histone marks at the IFN- γ locus [12], driving Th1 differentiation. In fact, conditional knockout of the GATA-3 gene reduces expression of Th2 cytokines in mice both *in vitro* and *in vivo* [13], and in isolated murine CD4⁺ lymphocytes [14]. Knockdown of GATA-3 expression using siRNA in human T cells also results in the loss of anti-CD3/CD28-mediated Th2 cytokine expression [15].

Glucosamine, a common constituent of the glycosaminoglycans in the cartilage matrix and synovial fluid, has been considered to have anti-inflammatory activity in articular cartilage and joint tissue [16]. In addition, immunoregulatory effects have been suggested. Glucosamine has been shown to interfere with the function of antigen-presenting cells and suppress the *in vitro* activation of dendritic cells (DC) [17, 18]. In fact, IFN- γ , a Th1 cytokine, and IL-5, a Th2 cytokine, have been inhibited by glucosamine in primary mixed lymphocyte cultures (MLC), which suggest that glucosamine could inhibit the activation of unprimed T cells [17]. Glucosamine has also been involved in immunoregulation after differentiation. Secretion of Th2 cytokines, IL-4 and IL-5, has been reduced after glucosamine was added to Th2-polarized MLC, but not IFN- γ in Th1-polarized MLC; these findings suggest additional effects associated with Th2 immune suppression.

NC/Nga mice, an inbred mouse strain, were the first mouse model of AD reported by Matsuda [19], and this strain has been used as an animal model for human AD [20]. NC/Nga mice have been reported to develop AD-like skin lesions under conventional conditions [21]. The elevation of serum levels of total IgE has been reported to correlate with the appearance of the AD-like lesion in NC/Nga mice, with massive infiltration of IL-4- and IL-5-producing CD4⁺ T cells, the degranulation of mast cells and eosinophils [22]. Patients with AD are highly sensitized to house dust mite (HDM) allergens based on the positive results from scratch and patch assay [23, 24]. A major species of HDM in Japan is Df, and its body and faeces are well known as major environmental allergens [25]. It has been reported that Df has the ability to induce AD-like skin lesion in NC/Nga mice when applied as a Df extract suspension [26].

The goal of this study was to investigate the effects of glucosamine on changes of immune responses in the development of Df-induced dermatitis of NC/Nga mice, a condition histologically and clinically similar to human AD [22]. The effects on the selective differentiation of naïve CD4⁺ T cells to Th1 and Th2 cells were evaluated. In addition, the immunological effects of glucosamine on peripheral blood mononuclear cells (PBMC) from patients with AD were examined. The results showed that glucosamine improved the symptoms associated with dermatitis, reducing Th2 cytokines and IgE as a result of suppression of the differentiation of naïve T cells to Th2 cells.

Materials and methods

Animals. Eight-week-old male NC/Nga mice were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) for this study. The mice were maintained in uncontrolled conventional Laboratory Animal

Facilities at the Dongguk University School of Medicine. The animal care and use committee of the research institute at Dongguk University Hospital approved all described studies.

Drugs and reagents. Glucosamine was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Df body ointment was prepared by Biostir Inc. (Kobe, Japan). One gram of Df body ointment contained 136.4-mg protein, 234- μ g Der f 1 and 7- μ g Der f 2.

Induction of AD in the NC/Nga mice. Induction of AD using Df body ointment was performed as described previously [26]. The hair on the backs of the NC/Nga mice was shaved using an electric shaver, followed by treatment with a skin-hair-remover (Niclean, Ildong, Korea). Barrier disruption was achieved by 4% sodium dodecyl sulphate treatment on the shaved dorsal skin and both surfaces of each ear three hours before Df body ointment (100 mg/mouse) application. These procedures were repeated twice a week for 4 weeks.

The administration of glucosamine on Df-induced NC/Nga mice. Glucosamine was administered intraperitoneally (i.p.) once a day for 4 weeks, starting after 1 week of Df application. The mice were divided into four groups according to the dose of glucosamine: 0 mg (control), glucosamine (5 mg), glucosamine (10 mg) and glucosamine (20 mg). For the control group, PBS was administered instead of glucosamine. Each group consisted of five mice.

Scoring of skin lesions. The extent of (1) erythema/haemorrhage, (2) scarring/dryness, (3) oedema, (4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate) and 3 (severe). The total skin score was defined as the sum of the individual scores [20].

Assay of the serum IgE concentration. Serum was collected at 1 week after the final administration. The concentration of total IgE in the serum was measured using an ELISA kit (Yamasa, Tokyo, Japan). The ELISA was performed in accordance with the manufacturer's instructions.

Assay of the cytokine production. One week after the final administration, a single-cell suspension was prepared from the spleen and incubated with 20 ng/ml of Phorbol 12-Myristate 13-Acetate (PMA; Sigma, St Louis, MO, USA) and 1 μ M of ionomycin (Calbiochem, La Jolla, CA, USA) at 37 °C in 5% CO₂ for 24 h. After the incubation period, the culture supernatant was collected. The concentrations of IL-4, IL-5 and IFN- γ were determined using the ELISA kit (R&D System, Minneapolis, MN, USA).

Histological analyses. Formalin-fixed and paraffin-embedded back skin samples from the mice were sliced and then stained with toluidine blue and Congo red to count the mast cells and eosinophils, respectively. The cell density was expressed as the number of cells per five high-power field ($\times 400$) for each section.

In vitro differentiation of mouse CD4⁺ T cells to Th2 and Th1 cells. Spleens from NC/Nga mice were removed

and erythrocyte-depleted single-cell suspensions were prepared in RPMI-1640 (Welgene Inc., Daegu, Korea) medium supplemented with 10% foetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, 100 µg/ml streptomycin (Welgene Inc.) and 0.05 mM 2-mercaptoethanol (Invitrogen). Splenic naïve CD4⁺ T cells from NC/Nga mice were purified using anti-CD4 magnetic microbeads and columns (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions at a purity >95%. The process of CD4⁺ T cell differentiation to Th2 and Th1 cells was carried out according to a previously described method [27], with minor modifications. To provide Th2 cell polarizing conditions, 2×10^5 cells/200 µl of CD4⁺

T cells were stimulated with 10 µg/ml anti-mouse CD3 antibody (BD biosciences, San Jose, CA, USA), 1 µg/ml anti-mouse CD28 antibody (BD biosciences), 10 ng/ml mouse IL-2 (BD biosciences), and 10 ng/ml mouse IL-4 (BD biosciences) in the presence or absence of D-glucosamine for 2 days in the above-mentioned RPMI-1640 on 96-well culture plates. The cultured T cells were then passaged and cultured for an additional 3 days on 24-well plates containing 10 ng/ml IL-2 and 10 ng/ml IL-4 in the presence or absence of glucosamine, but not anti-CD3 and anti-CD28. Under Th1 cell differentiation conditions, the T cells were cultured for 2 days in RPMI-1640 medium on 96-well culture plates containing anti-CD3 (10 µg/ml) and mouse IL-2 (10 µg/ml) as a Th2

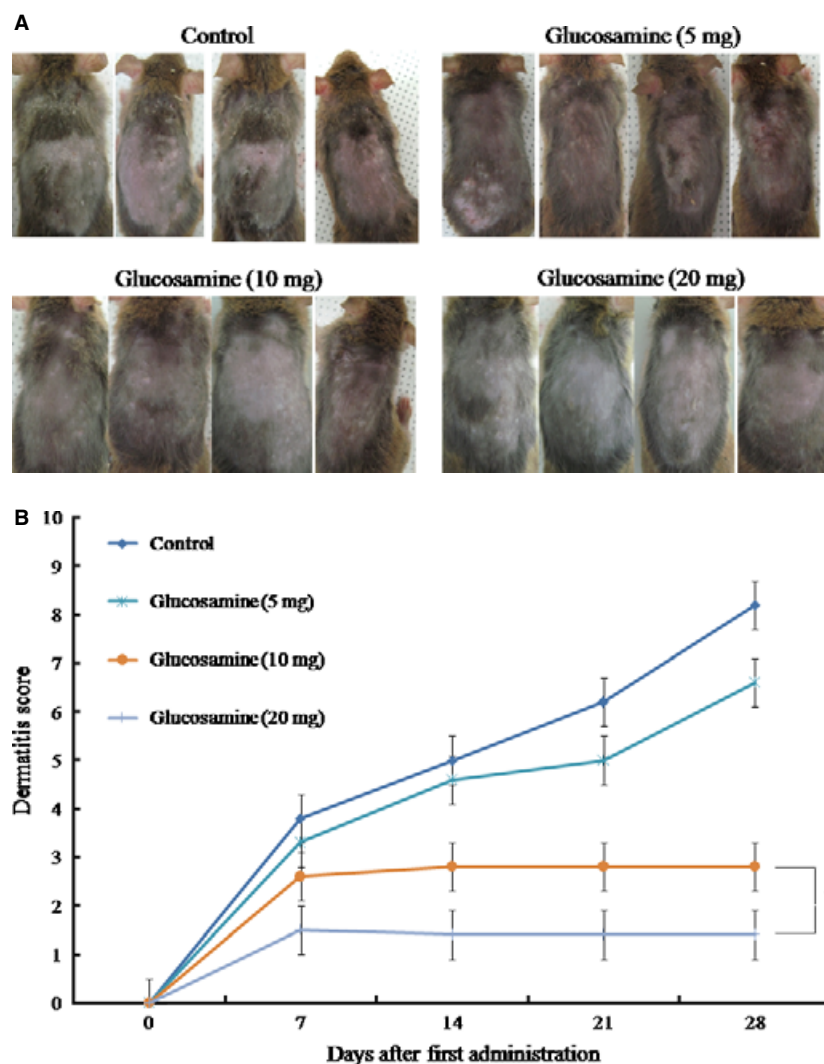


Figure 1 Effect of glucosamine on Df-induced atopic dermatitis (AD)-like skin lesions in NC/Nga mice. (A) Representative clinical features of Df-induced NC/Nga mice in the untreated (control) and treated groups with 5, 10, or 20 mg of glucosamine. The photograph was shown representative of four mice in each group. (B) The clinical skin score is the sum of individual scores based on the symptoms including erythema/haemorrhage, scarring/dryness, oedema and excoriation/erosion. Df-induced AD-like skin lesions in the NC/Nga mice in the untreated (control), or treated with 5, 10, or 20 mg of glucosamine intraperitoneally once a day for 4 weeks. The data represent the mean $\pm$ SD of five mice. * $P < 0.05$ when compared to untreated control mice (Student's *t*-test). The data shown are representative of two independent experiments.

polarization and mouse IL-12 (10 $\mu\text{g/ml}$). The cells were passaged and cultured for an additional 3 days on 24-well plates containing IL-2 and IL-12 in the presence or absence of glucosamine. For the measurement of IL-4, IFN- γ , GATA-3 and T-bet, the above polarized Th cells were stimulated with anti-CD3 for 24 h.

RT-PCR analysis of GATA-3 and T-bet mRNA. Total RNA from cells cultured under each condition was obtained with the Trizol Reagent (Invitrogen). Reversed transcription of the RNA was performed using the first-strand cDNA synthesis kit for RT-PCR (AMV; Boehringer Mannheim, Germany). The level of cytokine mRNA relative to GAPDH was measured by quantitative real-time PCR using the Light Cycler real-time PCR (Roche, Penzberg, Germany). The primers used were as follows: mouse GATA-3 5'-TTATCAAGCCCAAGCGAAG-3' (Forward) and 5'-AGACCGGGTCCCCATTAG-3' (Reverse); mouse T-bet 5'-GGACACCTCGGGAACACAGT-3' (Forward) and 5'-TCATGCTCACAGCTCGGAAC-3' (Reverse);

mouse GAPDH 5'-CTACCCCAATGTGTCCGTC-3' (Forward) and 5'-GCTGTTGAAGTCGCAGGAGAC-3' (Reverse).

Preparation of PBMC. PBMC were isolated from the blood of 12 patients with AD with a SCORAD more than 40, by Histopaque 1.077 (Sigma) density gradient centrifugation and resuspended at 2×10^6 cells/ml in RPMI-1640 (Welgene Inc.) and supplemented with 10% heat-inactivated foetal bovine serum (FBS; Welgene Inc.), 100 units/ml penicillin and 100 units/ml streptomycin (Invitrogen). The cells were stimulated by 20 ng/ml of PMA and 1 μM of ionomycin and cultured at different concentrations (1, 2, and 5 mM, respectively) of glucosamine for 24 h.

Statistical analysis. The statistical analysis of the experimental data was performed using the Student's *t*-test or Wilcoxon signed-rank test. The results are expressed as the mean $\pm$ SD. A *P*-value < 0.05 was considered significant.

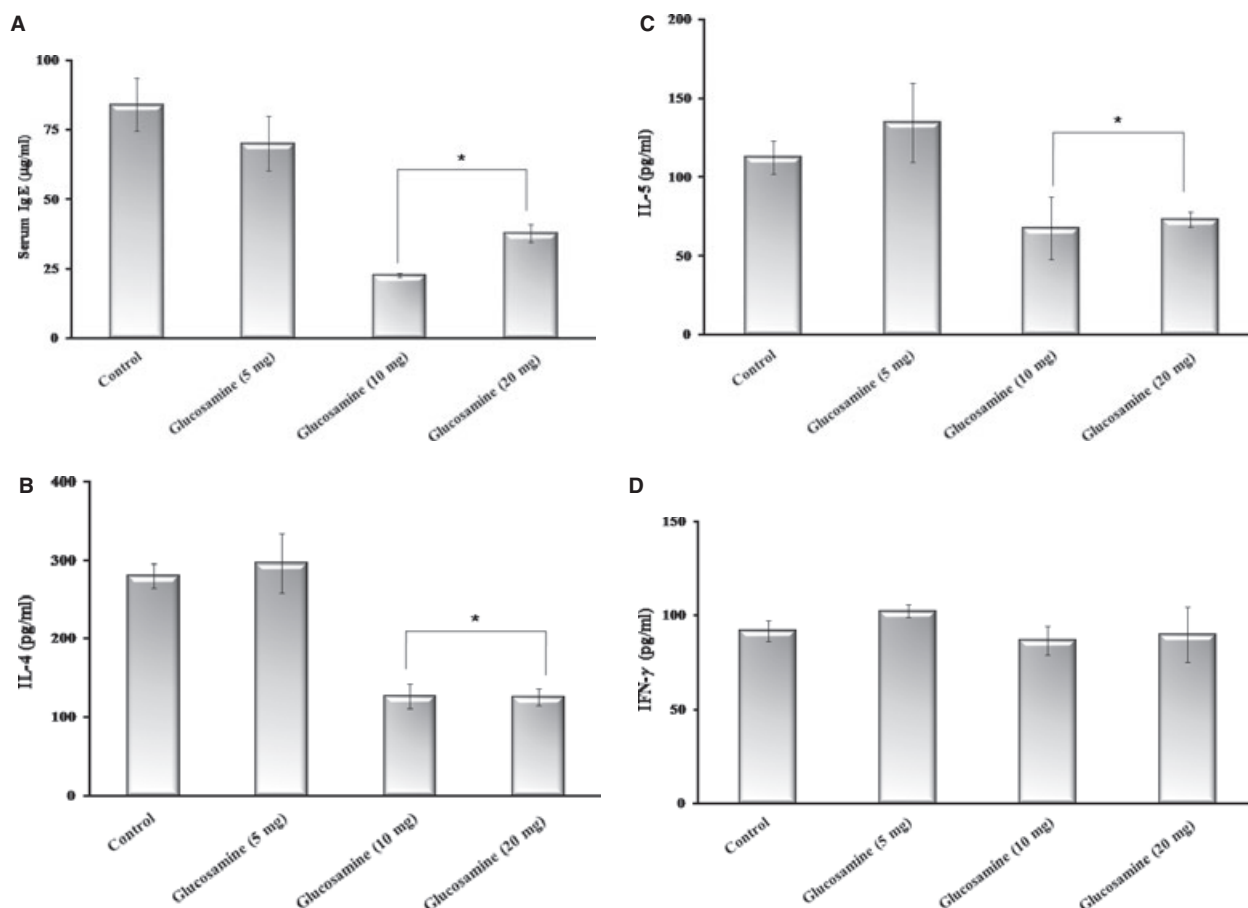


Figure 2 Serum IgE levels and cytokine production with glucosamine in the Df-induced atopic dermatitis-like skin lesions of NC/Nga mice. Mice serum was obtained from the eye. The concentration of IgE in mice serum was measured using a mouse IgE ELISA kit at 1 week after the final administration (A). Spleen cells were isolated from NC/Nga mice that had been left untreated (control), and treated with 5, 10, or 20 mg of glucosamine. The cells were stimulated with PMA and ionomycin. ELISA targeting IL-4 (B), IL-5 (C), and IFN- γ (D) was performed using the supernatant obtained 24 h after the stimulation. The data represent the mean $\pm$ SD of five mice. **P* < 0.005 when compared with untreated control mice (Student's *t*-test). The data shown are representative of two independent experiments.

Results

Effects of glucosamine on Df-induced AD-like skin lesions of NC/Nga mice

To examine the effects of glucosamine on Df-induced AD-like skin lesions in the NC/Nga mice *in vivo*, the NC/Nga mice were treated with different doses of glucosamine. The clinical symptoms including erythema/haemorrhage, scarring/dryness, oedema and excoriation/erosion were ameliorated or resolved in the groups treated with glucosamine (10 mg) and glucosamine (20 mg) (Fig. 1A),

with significant reduction in the clinical skin score ($P = 0.0023$, $P = 0.0018$, respectively) (Fig. 1B). However, the symptoms were unchanged in the glucosamine (5 mg) group (Fig. 1A), without a significant difference in the skin score between the glucosamine (5 mg) group and the control group (Fig. 1B).

Effects of glucosamine on Df-induced serum IgE and Th2 cytokine levels

Serum levels of IgE and Th2 cytokines were examined in the Df-induced NC/Nga mice after the administration of

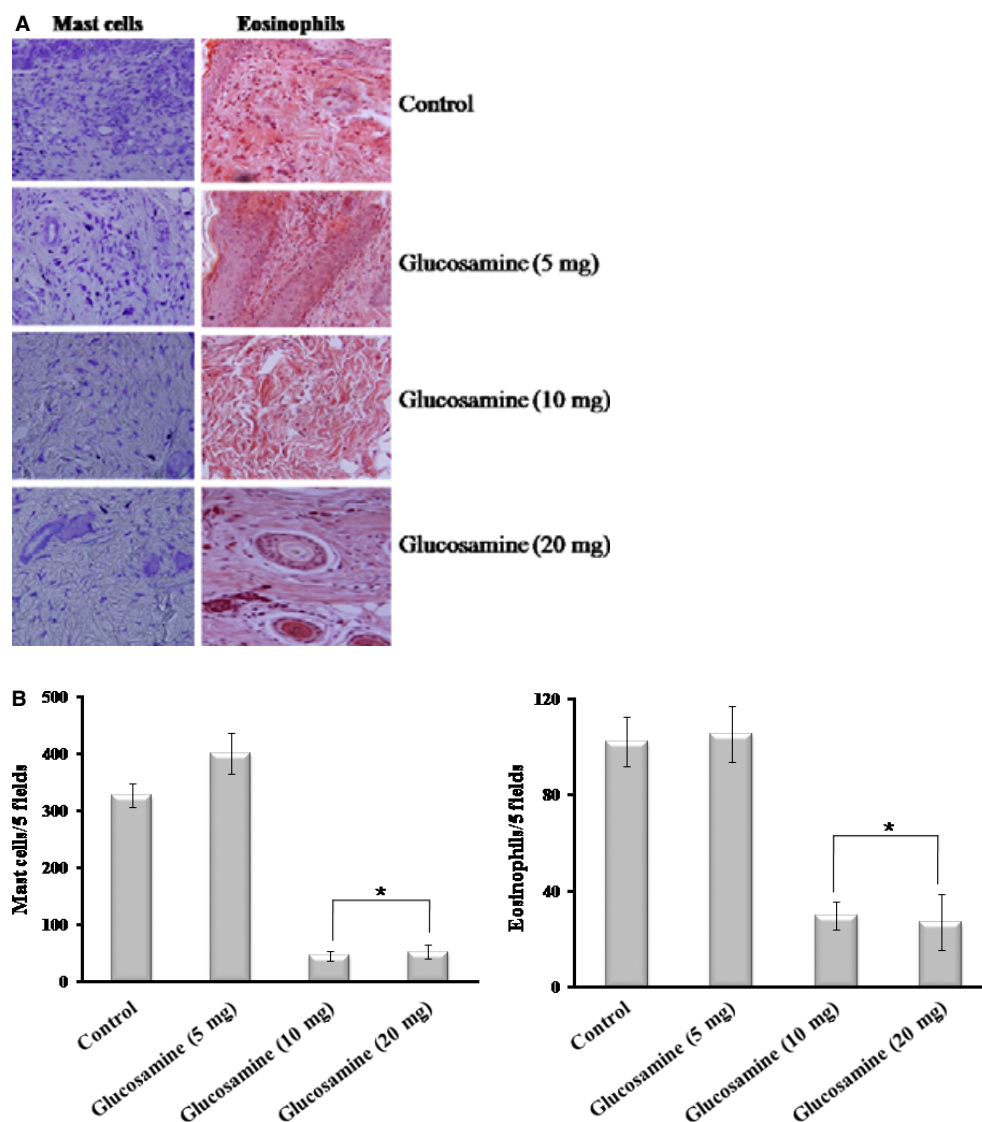


Figure 3 Histological analysis of the skin lesions. Skin samples from the back of NC/Nga mice were studied at 1 week after the final administration and fixed in 10% (v/v) buffered formalin, embedded in paraffin, section at 5 μ m. The sections were then stained with toluidine blue and Congo red for mast cells and eosinophils, respectively; (A) representative histological features in the Df-induced NC/Nga mice from the untreated (control), and treated groups with 5, 10, or 20 mg of glucosamine; (B) the cell density was expressed as the number of mast cells and eosinophils in five high-power fields ($\times 400$ for count) for each section. The data represent the mean $\pm$ SD of five mice. * $P < 0.005$ when compared with untreated control mice (Student's *t*-test). The data shown are representative of two independent experiments.

glucosamine. The total serum IgE levels were significantly decreased in the glucosamine (10 mg) groups and the glucosamine (20 mg) groups compared to the control group ($P = 0.002$ and $P = 0.003$, respectively) (Fig. 2A); however, there was no difference found between the glucosamine (5 mg) group and the control group (Fig. 2A). The expression levels of both IL-4 (Fig. 2B) and IL-5 (Fig. 2C) showed marked inhibition in the glucosamine (10 mg) and the glucosamine (20 mg) groups, compared to the glucosamine (5 mg) ($P = 0.008$ and $P = 0.011$, respectively) and the control groups ($P = 0.001$ and $P = 0.001$, respectively). No difference, however, was observed in IFN- γ production among all four groups (Fig. 2D).

Effect of glucosamine on skin infiltration of mast cells and eosinophils

To investigate whether glucosamine decreased infiltration of these inflammatory cells into the skin of the Df-induced NC/Nga mice, tissue sections were stained with toluidine blue and Congo red. The number of infiltrated cells, both mast cells and eosinophils (Fig. 3A,B), was significantly reduced in the glucosamine (10 mg) and glucosamine (20 mg) groups, compared to the mice that received glucosamine (5 mg) ($P = 0.003$ and

$P = 0.002$, respectively) and the control mice ($P = 0.001$ and $P = 0.002$, respectively).

Th1 and Th2 polarization in the naïve CD4⁺ T cells

Splenic CD4⁺ T cells, from the NC/Nga mice cultured with IL-4 and anti-CD3 antibody, produced IL-4, a Th2 cytokine, by the final anti-CD3 antibody stimulation (Fig. 4A). By contrast, the splenic CD4⁺ T cells cultured with IL-12 and anti-CD3 antibody produced IFN- γ , a Th1 cytokine, by the final anti-CD3 antibody stimulation (Fig. 4C). The mRNA levels of GATA-3 and T-bet, the master genes for Th2 and Th1 cells, respectively, closely paralleled the profile of production of each cytokine (Fig. 4B,D).

Effect of glucosamine on the differentiation of naïve CD4⁺ T cells to Th1 and Th2 cells

To investigate whether glucosamine was associated with selective differentiation of naïve CD4⁺ T cells to Th1 and Th2 cells, naïve CD4⁺ T cells from the spleen of NC/Nga mice were cultured with glucosamine for 5 days. The addition of glucosamine (0.5–2 mM) to the Th2 polarizing conditions significantly decreased the

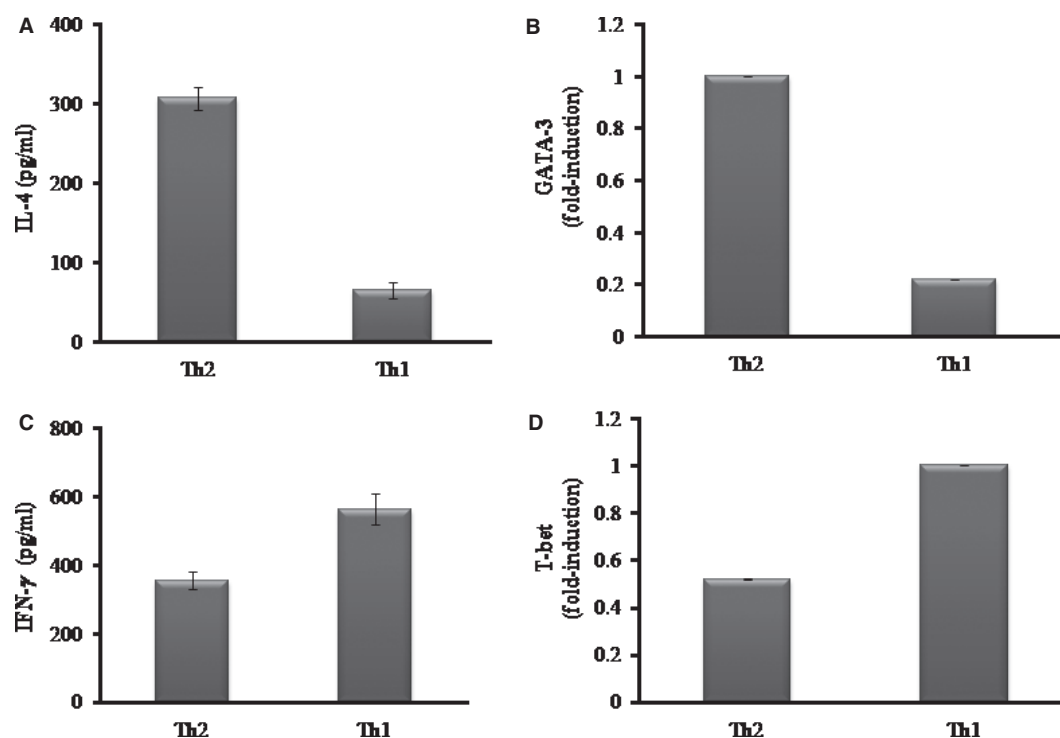


Figure 4 Th1 and Th2 polarization from naïve CD4⁺ T cells. The production of IL-4 (A) and IFN- γ (C), and the expression of GATA-3 (B) and T-bet (D) mRNA on T cells stimulated with anti-mouse CD3 antibody. To provide Th1 and Th2 polarization conditions, naïve CD4⁺ T cells purified from the NC/Nga mice and polarized under Th1 (IL-2 and IL-12) or Th2 (IL-4 and IL-2) conditions. Cultured T cells were stimulated with anti-mouse CD3 antibody. The supernatant was collected to measure the levels of cytokines by ELISA. The levels of T-bet and GATA-3 gene expression were determined by RT-PCR. Data are shown as the mean $\pm$ SD of the results from three separate experiments.

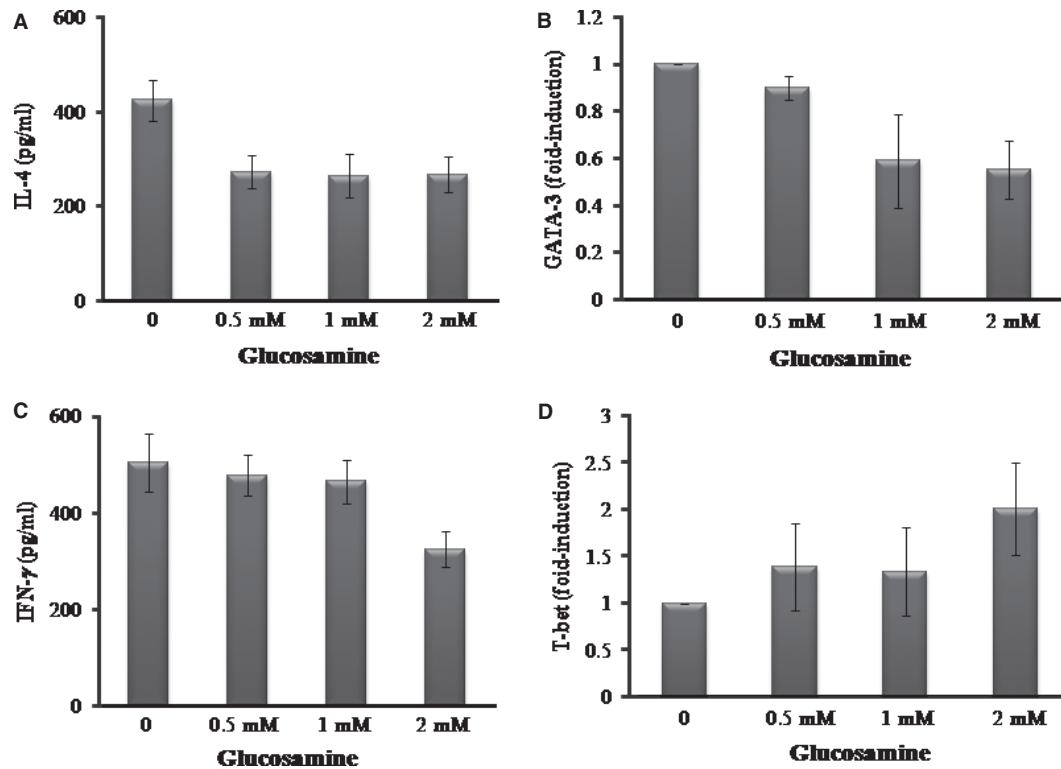


Figure 5 Effect of glucosamine on Th cell differentiation. The production of IL-4 (A) and IFN- γ (C), and the expression of GATA-3 (B) and T-bet (D) mRNA of T cells stimulated with anti-mouse CD3 antibody. To promote a selective differentiation pathway, naïve CD4⁺ T cells were purified from NC/Nga mice and polarized under Th1 (IL-2 and IL-12) or Th2 (IL-4 and IL-2) conditions in the presence or absence of glucosamine (0.5–2 mM). The supernatant was collected to measure the levels of the cytokines by ELISA. The levels of T-bet and GATA-3 gene expression were determined by RT-PCR. Data are shown as the mean $\pm$ SD of the results from three separate experiments.

levels of both IL-4 protein and GATA-3 mRNA (Fig. 5A,B). By contrast, under Th1 polarizing conditions, <2 mM of glucosamine was not adequate for the reduction in IFN- γ levels (Fig. 5C). Moreover, the levels of T-bet expression were not affected by up to 2 mM of glucosamine (Fig. 5D).

Effect of glucosamine on the cytokines in the PBMC of patients with AD

Glucosamine inhibited IL-4 and IL-5 cytokines in the PBMC from two AD patients in a dose-dependent manner, which was observed during trials with different doses (1, 2, and 5 mM) of glucosamine (Fig. 6A). Therefore, 5 mM of glucosamine was chosen for the study. Glucosamine significantly suppressed both IL-4 ($P = 0.007$) and IL-5 ($P = 0.002$) (Fig. 6B), but not IFN- γ (data not shown) in the PBMC from 12 patients with AD.

Discussion

Glucosamine has been shown to have immunosuppressive properties; however, its effects on the immunological responses in AD are not known. In this study, the effect

of glucosamine on AD was examined using NC/Nga mice with topical Df-induced dermatitis, an animal model for AD [20]. Glucosamine ameliorated, in a dose-dependent fashion, the clinical symptoms; however, a dose as low as 5 mg was not effective. The administration of doses of glucosamine that had clinical effects reduced Th2 cytokines such as IL-4 and IL-5, but not Th1 cytokines, IFN- γ . No significant effect on the Th1 cytokines might have been attributed to an insufficient dose of glucosamine; higher dose of glucosamine were required to reduce the levels of IFN- γ expression *in vitro* under polarization conditions. Similar doses of glucosamine with reduced Th2 cytokines decreased the serum levels of total IgE. As IL-4 is important in the development of Th2 cells and in changes to the IgE isotype [28], the reduction in IgE levels might result from suppression of the Th2 response. For each parameter, we have repeated the experiment once and found a similar type of profile, indicating the results are reproducible (data not shown). In fact, the correlation between the elevation of serum levels of total IgE and the production of Th2 cytokines has been previously reported [19].

The main target organ of AD is the skin, with inflammatory cellular infiltrate at this site. Although the

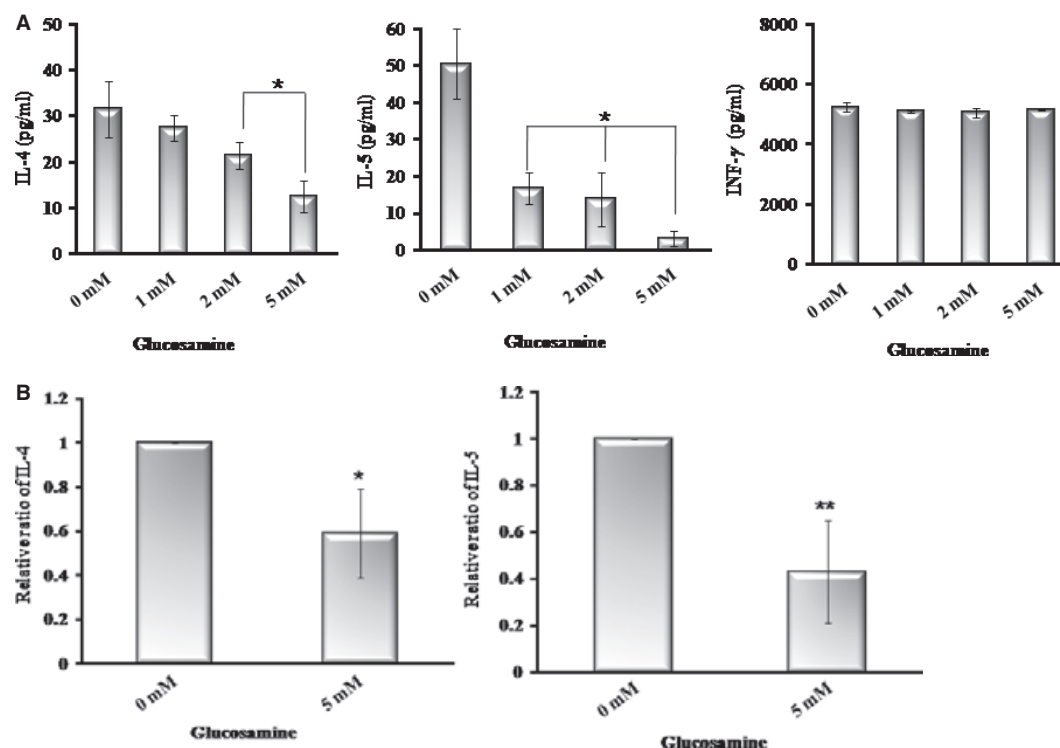


Figure 6 Production of Th2 cytokines in the glucosamine-treated peripheral blood mononuclear cells (PBMC) *in vitro* in patients with atopic dermatitis (AD). The PBMC from patients with AD were treated with various concentrations of glucosamine for 24 h after stimulation by 20 ng/ml of Phorbol 12-Myristate 13-Acetate (PMA) and 1 μ M of ionomycin. ELISA targeting of IL-4 and IL-5 was performed using the supernatants obtained 24 h after stimulation; (A) the optimal dose of glucosamine for the production of IL-4 and IL-5 in the PBMC of patients with AD; (B) the production of IL-4 and IL-5 cytokines in the PBMC from patients with AD after administration of glucosamine (5 mM) *in vitro*. The relative ratio of each cytokine was determined by calculating the ratio of each cytokine relative to the optical density of an untreated control. * $P < 0.05$ and ** $P < 0.005$ when compared to untreated control PBMC (Wilcoxon signed-rank test).

majority of cells are T cells, a large number of mast cells and DC accumulate in both the epidermis and the dermis of patients with AD [29]. In fact, mast cells have been shown to be involved in the differentiation of naïve DC to Th2-promoting DC by the secretion of several molecules [30, 31]. Moreover, mast cells are recognized as key effector cells in IgE-associated Th2-type immune responses, because they are activated by cross-linking of Fc ϵ RI. Therefore, the decrease in the mast cell infiltration may indicate an effect of glucosamine on Th2 inhibition. Th2 cytokines induce proliferation and activation of eosinophils in the skin. In fact, massive infiltration of eosinophils was found in Df-induced NC/Nga mice. The reduced eosinophilic infiltration associated with glucosamine suggested a suppressive effect of glucosamine on the Th2 immune response.

Th2-dominance is associated with AD. Control of the immune system dysregulation is required to be considered an effective immunomodulator. Naïve CD4⁺ T cells are thought to differentiate to either Th1 or Th2 cells, depending on the transcription factors and cytokine milieu [32]; IL-12 promotes the development of Th1 cells through STAT4 activation, whereas IL-4 promotes

Th2 differentiation with STAT6 [33]. Polarizing conditions for either Th1 or Th2 could be identified by each signature cytokine, IFN- γ or IL-4, and each master gene, T-bet or GATA-3 [12, 34], respectively. In fact, the treatment of naïve CD4⁺ spleen cells with IL-12 or IL-4 induced Th1 or Th2 polarization, respectively. Although glucosamine has been reported to be an immune modulator [17, 18], it has been unclear how glucosamine controls Th cell development. Using polarization conditions, the results of this study demonstrated that glucosamine inhibited Th2 differentiation; a lower dose of 0.5 mM could inhibit IL-4 expression. These results were not limited to the NC/Nga mouse model, and they were also observed in normal C57/BL6 mice (data not shown). Moreover, the reduction in IL-4 with glucosamine paralleled a decrease in the GATA-3 mRNA expression, suggesting a chain of processes [35]. However, no such correlation was observed between IFN- γ and T-bet in results treated with high dose glucosamine, suggesting different regulatory pathways for IFN- γ expression. In fact, the expression of IFN- γ might be regulated by other transcriptional factors such as the Ets-related molecule (ERM), nuclear factor (NF)- κ B, and NF of activated T

cells (NFAT) in addition to T-bet [36, 37]. Glucosamine has been reported to regulate NFAT and NF- κ B activation [38].

A predominance of Th2 cells in the peripheral blood samples of patients with AD has been previously reported [39]. Moreover, the PBMC, whenever stimulated by an allergen, have produced larger amounts of IL-4 with smaller amounts of IFN- γ compared to healthy controls [40]. As dominant Th2 immunomodulatory outcomes were observed in the *in vitro* and *in vivo* animal models, similar effects with glucosamine are expected in the PBMC of patients with AD. In fact, glucosamine treatment of the PBMC reduced the levels of IL-4 and IL-5, but not IFN- γ (data not shown) in the glucosamine-treated compared to untreated PBMC.

In conclusion, the results of this study demonstrated that glucosamine improved AD-like skin lesions in an NC/Nga mouse model by dominantly suppressing Th2 cells both *in vitro* and *in vivo*. Moreover, glucosamine reduced Th2 cytokine levels in the PBMC of patients with AD. These results suggest that glucosamine might be an effective immunomodulatory agent for the treatment of patients with AD.

Acknowledgment

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Conflicts of interest

None declared.

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