Inhibition of IL-32 Signaling by Bamboo Salt Decreases Pro-Inflammatory Responses in Cellular Models of Allergic Rhinitis

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ABSTRACT Previously, we showed the antiallergic effect of bamboo salt (BS) in allergic rhinitis (AR). We also demonstrated that interleukin (IL)-32 is an important mediator of AR. The aim of this study was to evaluate the effect and specific underlying mechanism of BS, NaCl, and the mineral mixture (components of BS other than NaCl, including zinc, magnesium, and potassium, Mix) on IL-32 signaling using the human monocyte cell line, THP-1. Here, we documented for the first time that BS significantly decreased IL-32-induced thymic stromal lymphopoietin protein and mRNA expression in THP-1 cells. BS treatment significantly inhibited IL-32-induced proinflammatory cytokine production including IL-1 β , IL-8, and tumor necrosis factor (TNF)- α by suppressing nuclear factor- κ B, p38 mitogen-activated kinase, and caspase-1 pathways. The presence of BS or Mix effectively suppressed IL-32-induced macrophage-like cell differentiation but NaCl exhibited no effect on monocyte-to-macrophage-like cell differentiation. In IL-32-induced macrophages, the production of IL-1 β , IL-6, IL-8, and TNF- α , and expression of inducible nitric oxide synthase and cyclooxygenase-2, induced by lipopolysaccharide was dramatically decreased in a dose-dependent manner after BS treatment. BS also significantly decreased IL-32-induced nitric oxide, IL-8, and TNF- α production. Furthermore, BS inhibited granulocyte-macrophage colony-stimulating factor-induced IL-32 and IL-8 protein and mRNA expression in EOL-1 cells. Taken together, BS suppressed inflammatory activity by inhibiting the IL-32 signaling pathway in AR.

KEY WORDS: • allergic rhinitis • bamboo salt • cyclooxygenase-2 • IL-32 • inducible nitric oxide synthase • thymic stromal lymphopoietin

INTRODUCTION

ALLERGIC RHINITIS (AR) IS A common nasal inflammatory disease characterized by symptoms of nasal itching, sneezing, nasal obstruction, congestion, and rhinorrhea. AR affects ~500 million people worldwide, with an especially high prevalence in industrialized nations.^{1,2} AR is widely recognized as a major cause of morbidity and mortality since it is a major risk factor for other inflammatory diseases such as asthma, rhinosinusitis, and allergic conjunctivitis, and affects quality of life by impairing sleep, academic and work performance, and recreational activities.^{3,4} AR is a type I

hypersensitive immune response caused by a series of cellular interactions. Initially, antigen presenting cells located in mucous take up and process environmental allergens and stimulate T helper 2 (Th2) cells to produce cytokines, which lead to IgE production from B cells. Subsequent allergen exposure induces mast cell degranulation and inflammation. Th2-induced cytokines and chemokines induce the AR reaction which is orchestrated by the infiltration of several immune cells, including eosinophils, basophils, neutrophils, and macrophages, into the injury site.⁴

Interleukin (IL)-32 is a newly discovered cytokine that plays a crucial role in inducing pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), IL-1 β , IL-6, and IL-8 through nuclear factor-kappa B (NF- κ B) and p38 mitogen-activated protein kinase (MAPK).⁵ Overexpression of IL-32 results in upregulated inducible nitric oxide synthase (iNOS) expression with subsequent NO production.⁶ IL-32 is induced by influenza A virus infection via COX-2 in the inflammatory cascade.⁷ The expression of IL-32 contributes

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to several inflammatory disorders and autoimmune diseases, including Crohn's disease, ulcerative colitis, rheumatoid arthritis, and chronic obstructive pulmonary disease and virus infection and cancer.⁸⁻¹² In addition, IL-32 regulates the differentiation of monocytes into macrophage-like cells through a caspase-3-dependent mechanism during host responses to infections.¹³ Recently, we demonstrated the relationship between IL-32 and AR using *in vitro* and *in vivo* model systems. IL-32 was significantly expressed in the nasal mucosa of AR patients, and functionally regulated the inflammatory cytokines and IgE production.¹⁴

Thymic stromal lymphopoietin (TSLP) consisting of a 4-helix bundle is a type I cytokine belonging to IL-2 family and has two distinct cognate receptors such as TSLP receptor (TSLPR) and IL-7Ra.¹⁵ TSLP is expressed and produced through caspase-1 and NF- κ B in mast cells.¹⁶ TSLPR is widely expressed on T cells, B cells, natural killer T cells, monocyte, macrophages, basophils, dendritic cells, and epithelial cells.^{17,18} The link of TSLP to atopic disease has attracted much attention in recent year because it induces Th2 differentiation by binding dendritic cells.¹⁹ Actually, patients with atopic dermatitis and asthma have been shown to highly express TSLP and moreover, patients with AR also produced TSLP in epithelial layer of nasal mucosa.²⁰⁻²³

Bamboo salt (BS) is a processed salt according to a traditional recipe using sun-dried salt and bamboo in Korea. When compared with crude salts, BS has lower toxicity and sulfate contents and higher contents of iron, silicon, potassium, and phosphate.^{24,25} Until now, extensive studies have shown various therapeutic effects of BS on diseases, including viral infections, diabetes, inflammatory disorders, gastropathy, and dental plaque.^{25,26} Of note, BS was very efficacious for treating AR, but the therapeutic mechanism by which BS regulate AR has not yet been elucidated.²⁷ In the present study, we demonstrated that BS regulated AR by inhibiting IL-32-induced TSLP production and macrophage differentiation in monocytes.

MATERIALS AND METHODS

Reagents

We purchased 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), biconchonic acid, lipopolysaccharide (LPS), and dimethyl sulfoxide (DMSO) from Sigma-Aldrich (St. Louis, MO, USA); human recombinant IL-32 γ from R&D Systems (Minneapolis, MN, USA); anti-human TSLP, TNF- α , IL-1 β , IL-6, IL-8 antibodies (Ab), biotinylated anti-human TSLP, TNF- α , IL-1 β , IL-6, and IL-8 Ab from BD Biosciences Pharmingen (San Diego, CA, USA); NF- κ B, actin, histone, p38, pp38, I κ B, tubulin, iNOS, and COX-2 Ab from Santa Cruz Biotechnology (Santa Cruz, CA, USA); CD11b and CD14 antibodies from eBioscience (San Diego, CA, USA).

Preparation of BS

BS was obtained from Tae-sung Food, Inc. (Jeonbuk, Republic of Korea). It was processed by a special technique,

with various durations of processing at extremely high temperature reaching about 1500°C with sun-dried salt, bamboo, pine tree firewood, pine resin, and yellow earth water. It contains mostly sodium chloride (about 91.7% of BS) along with potassium, calcium, iron, copper, manganese, sulfur, zinc, and magnesium in elementary quantities. Powdered BS was prepared by dissolving in distilled water (DW) then filtering it through 0.22 μ m syringe filter. Dilutions of BS were made in DW. Kim *et al.* reported the contents of zinc, magnesium, and potassium in the BS was higher compared with crude salt.²⁸ To compare with the ingredient except the salt of BS, we made a mineral mixture. Composition of mineral mixture consists of three minerals: zinc (65.2%), magnesium (23.6%), and potassium (11.2%).²⁷

Cells

THP-1 human monocyte cells obtained from the American Type Culture Collection (TIB-202; Manassas, VA, USA) and human EoL-1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified atmosphere in the presence of 5% CO₂.

Reverse transcription PCR analysis

We isolated the total RNA from THP-1 cells using an easy-BLUTM RNA extraction kit (iNtRON Biotechnology, Sungnam, Republic of Korea) according to the manufacturer's specification. Total RNA (2.5 μ g/mL) was heated at 65°C for 10 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 90 min at 37°C using a cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The PCR was performed with the following primer for human IL-1 β (5'-CCG GAT CCA TGG CAC CTG TAC GAT CA-3'; 5'-GGG GTA CCT TAG GAA GAC ACA AAT TG-3'); IL-8 (5'-CGA TGT CAG TGC ATA AAG ACA-3'; 5'-TGA ATT CTC AGC CCT CTT CAA AAA-3'); IL-32 (5'-TGA CAT GAA GAA GCT GAA GGC-3'; 5'-CAT GAC CTT GTC ACA AAA GCT C-3'); and GAPDH (5'-CAA AAG GGT CAT CAT CTC TG-3'; 5'-CCT GCT TCA CCA CCT TCT TG-3'). The annealing temperature was 62°C for IL-1 β , IL-8, IL-32, and GAPDH. Products were electrophoresed on a 2% agarose gel and visualized by staining with ethidium bromide.

Quantitative real-time PCR analysis

Quantitative real-time PCR was performed using a SYBR Green Master Mix and the detection of mRNA was analyzed using an ABI StepOne Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Primer sequences for the reference gene GAPDH and the genes of interest were as follows: GAPDH (5'-TCG ACA GTC AGC CGC ATC TTC TTT-3'; 5'-ACC AAA TCC GTT GAC TCC GAC CTT-3'); human TSLP (5'-CCC AGG CTA TTC GGA AAC TCA G-3'; 5'-CGC CAC AAT CCT TGT AAT TGT G-3'); human IL-1 β (5'-AAA CAG ATG AAG TGC TCC TT-3'; 5'-TGG AGA ACA CCA CTT GTT GC-3'); human CD11b (5'-ACG TAA ATG GGA CAA GCT G-3'; 5'-GAT CCT GAG GTT

CCG TGA AA-3'); human CD14 (5'-ACT TGC ACT TTC CAG CTT GC-3'; 5'-GCC CAG TCC AGG ATT GTC AG-3'). Typical profile times used were the initial step, 95°C for 10 min followed by a second step at 95°C for 15 s and 60°C for 30 s for 40 cycles with a melting curve analysis. The level of target mRNA was normalized to the level of the GAPDH and compared with the control. Data were analyzed using the $\Delta\Delta CT$ method.

Sandwich enzyme-linked immunosorbent assay

Cytokine levels in the culture supernatants were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (for TSLP, IL-1 β , IL-6, IL-8, and TNF- α assay; R&D Systems). Absorption of the avidin-horseradish peroxidase color reaction was measured

at 405 nm and compared with serial dilutions of human recombinants as a standard. All samples were performed in duplicate.

Direct ELISA

IL-32 levels in the culture supernatants were measured by a direct ELISA according to the manufacturer's protocol (R&D Systems). Absorption of the avidin-horseradish peroxidase color reaction was measured at 405 nm and compared with serial dilutions of human recombinants as a standard. All samples were performed in duplicate.

MTT assay

Cell viability was determined using an MTT assay. Briefly, 100 μ L of cell suspension (1×10^4 cells) was cultured in 96-well plates after pretreatment by each concentration of BS,

FIG. 1. BS inhibited the IL-32-induced production and mRNA expression of TSLP and IL-1 β . THP-1 cells (3×10^5) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 2 h and then stimulated with IL-32 (0.1 μ g/mL) for 24 h. The production of TSLP and IL-1 β in the supernatant was measured by the ELISA method (**A**, **B**). THP-1 cells (3×10^6) were treated with BS, NaCl, or Mix for 2 h and then stimulated with IL-32 for 5 h. The mRNA expressions of TSLP were measured by real-time PCR (**C**). The mRNA expressions of IL-1 β were measured by real-time PCR (lower) and RT-PCR (upper) (**D**). Cell viability was evaluated by an MTT assay (**E**). THP-1 cells were cultured in the presence of media, BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 24 h. The proliferation was measured with a BrdU incorporation assay (**F**). [#] $P < .05$; significantly different from the unstimulated cells value, ^{*} $P < .05$; significantly different from the IL-32-stimulated cells value. BS, bamboo salt; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TSLP, thymic stromal lymphopoietin.

NaCl, and Mix. The MTT solution (5 mg/mL) was added and the cells were incubated at 37°C for an additional 4 h. After washing the supernatant out, the insoluble formazan product was dissolved in DMSO. Then, the optical density was measured using an ELISA reader at 540 nm.

BrdU assay

Cell proliferation was determined using a colorimetric immunoassay based on the measurement of BrdU incorporated by DNA synthesis (Roche Diagnostics GmbH, Mannheim, Germany).

Caspase-1 enzymatic activity assay

Caspase-1 enzymatic activity was measured according to the manufacturer's instructions by using a caspase assay kit (R&D Systems).

Western blot analysis

The stimulated cells were lysed and separated through 10% SDS-PAGE. After electrophoresis, the protein was transferred to nitrocellulose membranes and then the membranes were blocked for 2 h with 1×PBST containing 5% skim milk. The primary antibodies (1:500 in PBST) were added and incubated overnight at 4°C. Afterward, the nitrocellulose membrane was washed five times for 15 min with PBST. For protein detection, the blot was incubated with secondary antibodies (1:3000 in PBST, rabbit for p38, NF- κ B, I κ B, iNOS, CD11b, and histone; mouse for pp38, tubulin, and CD14; goat for COX-2) conjugated with peroxidase for 40 min. Finally, the protein bands were visualized by an enhanced chemiluminescence assay purchased from Amersham Co. (Newark, NJ, USA) following the manufacturer's instructions.

Analysis of monocyte surface antigens by flow cytometry and confocal laser scanning microscopy

THP-1 cultured in the presence or absence of IL-32, BS, NaCl, and Mix for 6 days were washed in fluorescence-

activated cell sorter (FACS) buffer (phosphate buffered saline supplemented with 1% bovine serum albumin and 0.1% NaN₃) and then incubated with 2 μ L fluorescein isothiocyanate (FITC)-conjugated CD14 and phycoerythrin (PE)-conjugated CD11b antibodies for 30 min at 4°C. After washing with FACS buffer, cells were fixed with 0.01g/mL paraformaldehyde for 30 min and then stored in the dark until analyzed by flow cytometry. Cytofluorometry was performed with a FACScan (Becton Dickinson, Mountain View, CA, USA). All specimens were examined with a confocal laser scanning microscope.

Measurement of nitrite concentration

The differentiated macrophages (3×10^5) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 2 h and then stimulated with IL-32 (0.1 μ g/mL) for 48 h. NO synthesis in culture media was measured by a Griess assay method. To measure nitrite, 100 μ L aliquots were removed from conditioned medium and incubated with an equal volume of Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride/2.5% H₃PO₄) at room temperature for 10 min. The absorbance at 540 nm was determined by an automatic microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA). NO₂⁻ was determined by using sodium nitrite as a standard.

Statistical analysis

The experiments shown are a summary of the data from at least three experiments and are presented, as the mean $\pm$ standard error of the mean. Statistical evaluation of the results was performed by independent *t*-test and analysis of variance with Tukey *post hoc* test. The results were considered significant at a value of $P < .05$.

RESULTS

BS inhibited IL-32-induced TSLP and IL-1 β expression

In our previous study, we described that IL-32 induced TSLP and IL-1 β productions, thereby contributing

FIG. 2. BS inhibited IL-32-induced IL-8 and TNF- α production. THP-1 cells (3×10^5) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 2 h and then stimulated with IL-32 (0.1 μ g/mL) for 24 h. The production of IL-8 (A), TNF- α (B), and IL-6 (C) in the supernatant was measured using an ELISA method. [#] $P < .05$; significantly different from the unstimulated cells value, ^{*} $P < .05$; significantly different from the IL-32-stimulated cells value. TNF- α , tumor necrosis factor- α .

to rheumatoid arthritis and AR involvement, respectively.²⁹ Controlling IL-32-induced TSLP in AR, however, has not been defined yet. Thus, the present study sought to determine whether inhibiting IL-32-induced TSLP and IL-1 β production in THP-1 cells could be used a novel therapeutic target against AR. In addition, we investigated the effect of BS on this new target using ELISA, real-time PCR, and RT-PCR experiments. As shown in Figure 1A and B, increased TSLP and IL-1 β production by IL-32 were significantly decreased in a dose-dependent manner by BS treatment. Also, NaCl and Mix significantly decreased TSLP and IL-1 β production. The mRNA level of TSLP and IL-1 β induced by IL-32 was significantly decreased by BS, NaCl, or Mix (Fig. 1C, D). Similarly, the mRNA expression of IL-1 β was also significantly decreased by BS, NaCl, or Mix (Fig. 1D). BS had no effect on TSLP and IL-1 β production by itself (Fig. 1A, B). Cell toxicity and cell proliferation by BS, NaCl, or Mix was not observed (Fig. 1E, F).

BS inhibited IL-32-induced IL-8 and TNF- α production

IL-8 is a chemoattractant for eosinophil migration into inflammatory site and TNF- α plays an important role in promoting Th2 cytokine production. IL-32 significantly increased IL-8 and TNF- α production (Fig. 2A, B), whereas it had no effect on IL-6 production (Fig. 2C). Most of the cells treated with three different BS produced about 50% as much IL-8 compared with control. In addition, NaCl and Mix showed dramatically decreased IL-8 production. The induction of TNF- α production almost failed in cells treated with 0.01 mg/mL BS, however; cells treated with the other concentrations of BS displayed a higher percent inhibition. NaCl and Mix also resulted in decreased levels of TNF- α .

BS inhibited IL-32-induced NF- κ B, p38 MAP, and caspase-1 pathways

NF- κ B, p38 MAP, and caspase-1 pathways were required for the production of proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α in addition to chemokine, IL-8.⁵ Therefore, we tested whether BS blocked these signaling pathways and detected dose dependently decreased levels of phosphorylated p38 and activated NF- κ B in cells treated with BS (Fig. 3A, B); however, NaCl resulted in almost negligible effect on phosphorylated p38 and NF- κ B inhibition. For comparison, Mix decreased phosphorylated p38 and NF- κ B expression. Caspase-1, a third pathway activated by IL-32, plays a key role in converting of pro-IL-1 β and IL-18 into mature-IL-1 β and IL-18 form.³⁰ As shown in Figure 3C, the increased caspase-1 activity by IL-32 was decreased by BS and Mix treatment.

Effect of BS in IL-32-induced macrophage-like cells differentiation

Netea *et al.* reported that IL-32 induces the differentiation of monocytes into macrophages and our previous study also revealed that THP-1 cells differentiated into macrophage-

FIG. 3. BS inhibited the IL-32-induced p38, NF- κ B, and caspase-1 activations. THP-1 cells (3×10^6) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 2 h and then stimulated with IL-32 (0.1 μ g/mL) for 2 h. Phosphorylated p38 was determined by western blot analysis (A). NF- κ B in nuclear extract and I κ B α in cytoplasmic extract were determined by western blot analysis (B). THP-1 cells (3×10^6) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 2 h and then stimulated by IL-32 (0.1 μ g/mL) for 2 h. Caspase-1 activity was measured by using a caspase-1 assay kit (C). Results are representative of three independent experiments with duplicated samples. [#] $P < .05$; significantly different from the unstimulated cells value, ^{*} $P < .05$; significantly different from the IL-32-stimulated cells value. NF- κ B, nuclear factor-kappa B.

like cells following IL-32 stimulation.^{29,31} We therefore investigated whether BS could prevent the differentiation of THP-1 cells into macrophage-like cells. As shown in Figure 4A, BS significantly lowered the heightened CD11b and CD14 mRNA levels induced by IL-32. We also detected significant downregulation of CD11b and CD14 mRNA levels in cells treated with Mix. In contrast, NaCl failed to

FIG. 4. BS inhibited the IL-32-induced macrophage differentiation. THP-1 cells (3×10^5) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μg/mL) for 2 h and then stimulated with IL-32 (0.1 μg/mL) for 6 days. Real-time PCR of macrophage markers, CD11b and CD14 mRNA after stimulation of THP-1 cells (**A**). CD11b and CD14 proteins were determined by western blot analysis (**B**). FACS analysis of protein expression of macrophage markers, CD11b and CD14 (**C**). CD11b (red) and CD14 (green) were examined with confocal laser-scanning microscope (**D**). Results are representative of three independent experiments with duplicated samples. # $P < .05$; significantly different from the unstimulated cells value, * $P < .05$; significantly different from the IL-32-stimulated cells value. Blank, unstimulated cells. FACS, fluorescence-activated cell sorter. Color images available online at www.liebertpub.com/jmf

inhibit CD11b and CD14 mRNA expression. The CD11b mRNA inhibition rate of BS was higher than that of Mix. The protein expression of CD11b and CD14 was determined by western blot analysis. BS inhibited the expression of these proteins in a dose-dependent manner (Fig. 4B). We also performed a FACS analysis for CD11b and CD14 protein expression and found that the expression of CD11b and CD14 proteins that were increased by IL-32 were lowered by the treatment with BS and Mix, whereas NaCl had no effect on IL-32 induced macrophage-like cells differentiation (Fig. 4C, D). Confocal laser scanning microscopic analysis clearly demonstrated that the enhanced expression of CD11b and CD14 was induced by the treatment of IL-32, but it was markedly blocked by the treatment of BS (Fig. 4D).

Effects of BS on proinflammatory cytokine production and iNOS and COX-2 expression in macrophages

We evaluated whether BS inhibits proinflammatory cytokine production induced by LPS in macrophages. BS significantly decreased LPS-induced IL-1β, IL-6, IL-8, and TNF-α by LPS production, however, NaCl and Mix were less effective inhibitors (Fig. 5A). We determined whether the anti-inflammatory actions of BS were associated with inhibition of iNOS and COX-2. As shown in Figure 5B, protein expressions of iNOS and COX-2 were dramatically decreased in the presence of BS and Mix, but not NaCl. We also determined whether BS inhibits NO and proinflammatory cytokine production induced by IL-32 in macrophage. IL-32 significantly induced NO and proinflammatory cytokine

FIG. 5. BS inhibited the NO and proinflammatory cytokine production and iNOS and COX-2 expression in macrophage. THP-1 cells (3×10^7) were cultured with IL-32 ($0.1 \mu\text{g/mL}$) for 6 days. The differentiated macrophages (3×10^5) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 $\mu\text{g/mL}$) for 2 h and then stimulated with LPS. Produced IL-1 β , IL-6, IL-8, and TNF- α were measured by ELISA method (A). Protein expression of iNOS and COX-2 were determined by western blot analysis (B). The iNOS and COX-2 were quantitated by densitometry (C). The differentiated macrophages (3×10^5) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 $\mu\text{g/mL}$) for 2 h and then stimulated with IL-32 ($0.1 \mu\text{g/mL}$) for 48 h. Nitric oxide release was measured by the Griess (D). Proinflammatory cytokines were measured by an ELISA method (E). Results are representative of three independent experiments with duplicated samples. $^{\#}P < .05$; significantly different from the unstimulated cells value, $^*P < .05$; significantly different from the LPS (or IL-32)-stimulated cells value. iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide.

productions, but they were significantly decreased by BS (Fig. 5D, E, $P < .05$).

BS inhibited IL-32 and IL-8 expression in the human eosinophilic leukemia cell line EoL-1

Eosinophils are key effector cells contributing to the pathophysiology of AR and GM-CSF is an activator of eosinophils. We observed that the increased IL-32 and IL-8 protein production and mRNA expression by GM-CSF was significantly decreased with treatment of BS, NaCl, and Mix (Fig. 6A–C).

DISCUSSION

AR is mediated by a series of cellular interactions in a cascade of events including early and late phase responses. Antigen-presenting cells including monocytes/macrophages and dendritic cells predominantly located in the nasal mucosa surface take up common environmental allergens, process them into short peptides, and present the processed peptides to Th2 cells by using an MHC class II molecule on their surface.^{32–34} In early phase response, activated mast cells produce preformed mediators, which cause symptoms of AR and infiltration of inflammatory cells.³⁵ In late phase

FIG. 6. BS inhibited the GM-CSF-induced IL-32 and IL-8 production and mRNA expression in EoL-1. EoL-1 cells (3×10^5) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 2 h and then stimulated with GM-CSF (10 ng/mL) for 24 h. IL-32 production was measured by an ELISA method (A). IL-8 production was also measured by an ELISA method (B). EoL-1 cells (5×10^6) were treated with BS (0.01, 0.1, and 1 mg/mL), NaCl (1 mg/mL), or Mix (3 μ g/mL) for 2 h and then stimulated with GM-CSF (10 ng/mL) for 4 h. The mRNA expressions of IL-32 and IL-8 were analyzed by RT-PCR (C). [#] $P < .05$; significantly different from the unstimulated cells value, ^{*} $P < .05$; significantly different from the GM-CSF-stimulated cells value.

response, the influx of monocytes/macrophages originating from bone marrow contributes to continued nasal inflammation, since they produce diverse proinflammatory cytokines.^{36–38} Furthermore, macrophages cause bronchial hyperresponsiveness by releasing bronchoconstrictor, O_2^- radicals, and nitric oxide.^{39,40}

TSLP is an extremely important factor for the development of allergic disorder since it promotes Th2 differentiation and Th2 cytokine production preferentially. It is reported that TSLP is predominantly expressed in epithelial cells and mast cells bind to its heterodimeric receptor, TSLPR and IL-7Ra, on dendritic cells. Then, it promotes the Th2 response by upregulating OX40L expression, which is an essential costimulatory mediator, on naïve T cells.^{23,41} IL-32-induced TSLP production in monocytes plays a critical role in etiology of rheumatoid arthritis.²⁹ Therefore, we supposed that inhibiting IL-32-induced TSLP production could be a novel and powerful therapeutic target for AR, since monocyte/macrophages, IL-32, and TSLP also are key factors for AR. When we treated IL-32-stimulated THP-1 cells with BS, NaCl, and Mix, the production of TSLP was significantly decreased. In addition, BS, NaCl, and Mix inhibited the production of proinflammatory cytokines including IL-1 β , IL-8, and TNF- α in THP-1 cells. NF- κ B and p38 MAPK are known to be responsible for the production of TNF- α , IL-1 β , IL-6, and IL-8. Moreover, IL-32 also promotes IL-1 β and IL-6 production by activating caspase-1.^{5,42} Consistent with this mechanism, BS, NaCl, and Mix also controlled the proinflammatory cytokine production via NF- κ B, p-38 MAPK, or caspase-1 pathways.

During the differentiation of monocytes into macrophages, the expression of CD11b and CD14 is upregulated.²⁹ BS and Mix significantly inhibited the differentiation of THP-1 cells into macrophage-like cells. By contrast, NaCl was not able to inhibit macrophage differentiation. This indicates that Mix is active component of BS responsible for the differentiation of macrophages. This result also indicated that significant differences between BS and NaCl may exist in the mechanisms and regulation of macrophage differentiation. Further study is needed to assess the distinct mechanism between them.

The chronic inflammatory response of AR is caused by the overproduction of proinflammatory cytokines, prostaglandin E2 (PGE₂), and nitric oxide (NO) by macrophages. The iNOS generates NO, and COX-2 is required for prostaglandins, prostacyclin, and thromboxane. Suppressing the expression of iNOS and COX-2 has been regarded as a therapeutic target for treating inflammation. BS inhibited the production of proinflammatory cytokines in macrophage-like cells, and the expression of iNOS and COX-2. These results suggest that BS may exert an anti-inflammatory effect in AR.

Eosinophils are innate effector cells that contribute to the pathology associated with allergic inflammatory conditions. Their recruitment to inflammatory sites occurs in response to chemotactic and activation signals, such as eotaxin and IL-5, and is a tightly controlled process.⁴³ Several cytokines are known to influence eosinophil function. In particular,

GM-CSF is a major survival and activating factor for hematopoietic cells that primes mature macrophages, eosinophils, and neutrophils and is known as a pleiotropic and proinflammatory cytokine.⁴⁴ GM-CSF increased the inflammatory reaction through the intracellular pathway such as IL-32.¹⁴ In this study, we showed that BS reduced the GM-CSF-induced IL-32 production and mRNA expression in EoL-1 cells. Taken together, these reports indicate that BS may be an important regulator of the inflammation of AR.

In conclusion, we demonstrated that BS inhibits IL-32-induced TSLP production and inflammatory cytokine production via p38 MAP, NF- κ B, and caspase-1 pathways. Furthermore, BS inhibits IL-32-induced differentiation of THP-1 cells into macrophage-like cells and IL-32 expression in EoL-1 cells. Our results provide convincing evidence that BS may have efficacy for alleviating inflammation associated with AR.

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AUTHOR DISCLOSURE STATEMENT

The authors have declared that no competing interests exist.

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