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Cysteine ameliorates allergic inflammatory reactions by suppressing thymic stromal lymphopoietin production in activated human mast cells

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ABSTRACT

Thymic stromal lymphopoietin (TSLP) derived by mast cells is recognized as a critical factor in many allergic inflammatory disorders. Cysteine is a well-known amino acid which exhibits anti-inflammatory activities. However, the effect and mechanism of cysteine on TSLP production have not been investigated. Thus, we hypothesized that cysteine may regulate TSLP production from mast cells. To test this hypothesis, the anti-inflammatory effects and signaling pathways of cysteine were investigated in phorbol 12-myristate 13-acetate 4 and calcium ionophore A23187 (PMACI)-stimulated human mast cell line HMC-1. Cysteine dramatically attenuated the levels of TSLP of both mRNA and protein without cytotoxicity. Moreover, cysteine suppressed caspase-1 activation and nuclear factor- κ B translocation. The phosphorylation of p38 and c-Jun N-terminal kinase was downregulated in all cases in PMACI-stimulated HMC-1 cells treated with cysteine. In addition, cysteine decreased PMACI-induced proinflammatory cytokines in terms of both protein and mRNA levels. In conclusion, cysteine regulates TSLP production by blocking caspase-1, nuclear factor- κ B, p38, and c-Jun N-terminal kinase-dependent pathways in activated HMC-1 cells, suggesting its potential as a regulator of allergic inflammatory diseases.

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1. Introduction

Allergic rhinitis (AR), the most common nasal inflammatory disorder affecting 500 million people around the world, is characterized by sneezing, itching, lacrimation, and rhinorrhea

[1]. In fact, the global incidence of AR is increasing, and it imposes a considerable economic and quality-of-life burden to patients and society. Consequently, there is growing interest in the search for novel and long-acting therapeutic agents.

Abbreviations: Abs, antibodies; ANOVA, analysis of variance; AR, allergic rhinitis; BCA, bichinchonic acid; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated protein kinase; GADPH, glyceraldehyde 3-phosphate dehydrogenase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF- κ B, nuclear factor- κ B; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate; PMACI, phorbol 12-myristate 13-acetate 4 and calcium ionophore A23187; RT-PCR, reverse transcription-polymerase chain reaction; TNF- α , tumor necrosis factor- α ; TSLP, thymic stromal lymphopoietin.

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Thymic stromal lymphopoietin (TSLP) is primarily induced in the epithelium of various organs, such as the skin, gut, and lung in response to diverse allergens. TSLP is known to contribute to the pathogenesis of allergic inflammatory disorders such as chronic obstructive pulmonary disease, atopic dermatitis, and asthma [2]. Recent evidence has demonstrated increased TSLP expression levels in the nasal epithelium of patients diagnosed with AR, and earlier studies have found significantly increased TSLP expression levels in vitro and in murine AR models [3–6]. Epithelial cell-derived TSLP has a profound influence on the secretion of tumor necrosis factor (TNF)- α [7]. Moreover, TSLP acts as a critical molecule in the proliferation of mast cell [8]. Mast cells are responsible for allergic inflammatory responses in the clinical phase because they secrete a diverse range of mediators, such as serglycin proteoglycans, biogenic amines, and proinflammatory cytokines, such as TSLP, interleukin (IL)-1 β , IL-6, TNF- α , and IL-8, when mast cells are sensitized by the binding of multivalent allergens [9–11].

Cysteine, a semiessential amino acid, contributes to protein metabolism and serves as a substrate for protein synthesis such as glutathione, which has high antioxidative potency [12]. For this reason, cysteine can protect several tissues and organs from free oxidative radicals. In addition, supplementation with cysteine has shown anti-inflammatory properties by reducing IL-1 β and TNF- α in an animal model of colitis [12–14]. Recently, Hasegawa et al [15] demonstrated that cysteine exhibits anti-inflammatory properties in human monocytes. However, the effect and mechanism of cysteine on TSLP production have not been investigated. Thus, we hypothesized that cysteine regulates TSLP production from mast cells. To test this hypothesis, the antiallergic inflammatory effects of cysteine were investigated in phorbol 12-myristate 13-acetate and calcium ionophore A23187 (PMACI)-stimulated human mast cell line HMC-1. In addition, we investigated its signaling pathways to provide further clues regarding the antiallergic inflammatory effects of cysteine. In this study, we observed that cysteine regulates TSLP production by blocking caspase-1, nuclear factor (NF)- κ B, p38, and c-Jun N-terminal kinase (JNK)-dependent pathways in activated HMC-1 cells, suggesting that it might be a potential regulator of allergic inflammatory diseases.

2. Methods and materials

2.1. Materials

Cysteine (minimum 98% pure), phorbol 12-myristate 13-acetate (PMA, protein kinase C activator), calcium ionophore (A23187), dimethyl sulfoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bovine serum albumin, phosphate-buffered saline (PBS), avidin peroxidase, and bicinchoninic acid (BCA) were purchased from Sigma Chemical Co (St Louis, MO, USA). Isocove's modified Dulbecco's medium, streptomycin, fetal bovine serum, and penicillin were from Gibco BRL (Grand Island, NY, USA). Anti-human TSLP/IL-6/IL-1 β /TNF- α /IL-8 antibodies (Abs), biotinylated anti-human TSLP/IL-6/IL-1 β /TNF- α /IL-8 Abs, and recombinant

human TSLP/IL-6/IL-1 β /TNF- α /IL-8 Abs were purchased from BD Biosciences Pharmingen (San Diego, CA, USA). Abs for extracellular signal-regulated protein kinase (ERK), phosphorylated (p)-ERK, JNK, p-JNK, p38, pp-38, NF- κ B, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), poly(ADP-ribose) polymerase, and caspase-1 were obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). The caspase-1 assay kit was supplied by R&D Systems, Inc (Minneapolis, MN, USA).

2.2. Cell culture and stimulation

HMC-1 cells were cultured at 37°C in 5% CO₂ and 95% humidity in Isocove's modified Dulbecco's medium supplemented with 100 U/mL penicillin, 100 μ g/mL streptomycin, 10 μ mol/L monothioglycerol, and 10% heat-inactivated fetal bovine serum. Cysteine was dissolved in distilled water. Cells were treated with cysteine for 1 hour prior to stimulation with 20 nmol/L of PMA plus 1 μ mol/L of A23187 (PMACI) and incubated at 37°C for various times.

2.3. Enzyme-linked immunosorbent assay

The cytokines were measured by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (BD Bioscience and Pharmingen). Absorption of the avidin-peroxidase color reaction was measured at 405 nm and compared with serial dilutions of human recombinants as a standard.

$$\% \text{ Inhibition} = (a-b) \times 100/a,$$

where a is cytokine secretion without cysteine and b is cytokine secretion with cysteine.

All samples were performed in duplicate.

2.4. Reverse transcription-polymerase chain reaction

HMC-1 cells were pretreated by cysteine (0.1, 1, or 10 μ g/mL) for 1 hour and triggered by PMACI. RNA was isolated from the cells using an easy-BLUE RNA extraction kit (iNtRON Biotechnology, Kyunggi-do, Korea) according to the manufacturer's instructions. Total RNA in the final elutes was quantitated using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). A total of 2.5 μ g RNA was heated at 75°C for 5 minutes and then chilled on ice, and cDNA was synthesized using a cDNA synthesis kit (Bioneer Corporation, Daejeon, Republic of Korea). Primers for polymerase chain reaction (PCR) are shown in Table 1. GAPDH was used to verify whether equal amounts of RNA were used for PCR amplification from different experimental conditions. Saturation curves for PCR were obtained from various experimental conditions (RNA concentrations, annealing temperatures, and PCR cycle numbers). The optimal amplification conditions (annealing temperature and PCR cycle number) of primers for the PCR were determined. The annealing temperature was 62°C for human TSLP; 50°C for human IL-6, IL-1 β , TNF- α , and IL-8; and 60°C for human GAPDH, respectively. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

Table 1 – List of primers used for RT-PCR

Primers	Sequence
TSLP forward	5'-TAT GAG TGG GAC CAA AAG TAC CG-3'
TSLP reverse	5'-GGG ATT GAA GGT TAG GCT CTG G-3'
IL-1 β forward	5'-CCG GAT CCA TGG CAC CTG TAC GAT CA-3'
IL-1 β reverse	5'-GGG GTA CCT TAG GAA GAC ACA AAT TG-3'
TNF- α forward	5'-CAC CAG CTG GTT ATC TCT CAG CTC-3'
TNF- α reverse	5'-CGG GAC GTG GAG CTG GCC GAG GAG-3'
IL-6 forward	5'-GAT GGATGC TTC CAATCT GGAT-3'
IL-6 reverse	5'-AGT TCT CCATAG AGA ACA ACA TA-3'
IL-8 forward	5'-CGA TGT CAG TGC ATA AAG ACA-3'
IL-8 reverse	5'-TGA ATT CTC AGC CCT CTT CAA AAA-3'
GAPDH forward	5'-CAA AAG GGT CAT CAT CTC TG-3'
GAPDH reverse	5'-CCT GCT TCA CCA CCT TCT TG-3'

2.5. MTT assay

Cell viability was assessed by MTT assay. MTT assay was performed according to our previous report [8]. HMC-1 cells suspension was cultured in 24-well plates for 24 hours after treatment by each concentration of cysteine. A total of 20 μ L of MTT solution (5 mg/mL) was added, and the cells were incubated at 37°C for 4 hours. The insoluble formazan product was dissolved in dimethyl sulfoxide after washing the supernatant. Then, optical density of 96-well culture plates was measured using an ELISA reader at 540 nm.

2.6. Caspase-1 assay

HMC-1 cells were treated with cysteine (0.1, 1, or 10 μ g/mL) for 1 hour before stimulation with PMACI incubated for 2 hours. Caspase-1 activity was measured according to the manufacturer's specifications using a caspase-1 assay kit (R&D Systems, Inc). Equal amounts of the total protein were quantified by a BCA protein quantification kit in each lysate. Catalytic activity of caspase-1 from the cell lysate was measured by the proteolytic cleavage of YVAD-pNA (BioVision, Inc, San Francisco, CA, USA) for 4 hours or at 37°C. The plates were read at 405 nm.

2.7. Preparation of nuclear and cytosolic extracts

Isolation of nuclear and cytosolic extracts was performed according to our previous report [16]. Briefly, after activating the cells for the times indicated, cells were washed in ice-cold PBS and centrifuged at 15 000g for 1 minute. Cells were resuspended in 40 μ L of a cold hypotonic buffer (10 mmol/L HEPES/KOH, 2 mmol/L MgCl₂, 0.1 mmol/L EDTA, 10 mmol/L KCl, 1 mmol/L DTT, and 0.5 mmol/L PMSF, pH 7.9) on ice for 15 minutes. Cells were gently lysed with 2.5 μ L of 10% Nonide P-40 and then centrifuged at 15 000g for 3 minutes at 4°C. The supernatant was aliquots (cytosolic protein), and the pellets were gently resuspended in 40 μ L of cold saline buffer (50 mmol/L HEPES/KOH, 50 mmol/L KCl, 300 mmol/L NaCl, 0.1 mmol/L EDTA, 10% glycerol, 1 mmol/L DTT, and 0.5 mmol/L PMSF, pH 7.9) and then left on ice for 20 minutes. After conducting the centrifugation (15 000g for 15 minutes at 4°C), the aliquots of supernatant containing the nuclear proteins were

stored in liquid nitrogen until ready for analysis. Finally, BCA protein assay was used to measure protein concentrations.

2.8. Western blot analysis

Western analysis was performed according to our previous report [8]. For the Western blot analysis, cell extracts were prepared by a detergent lysis procedure. Samples were heated at 95°C for 5 minutes and briefly cooled on ice. After the centrifugation at 15 000g for 5 minutes, 50- μ g aliquots were subjected to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then electrotransferred to nitrocellulose membranes in 25 mmol/L Tris, pH 8.5, 200 mmol/L glycerin, and 20% methanol at 25 V. The membrane was blocked for at least 2 hours with PBS with 0.05% Tween 20 and then probed with primary Abs for 1 hour at room temperature. The membrane was treated with peroxidase-conjugated secondary Abs, and proteins were visualized by enhanced chemiluminescence detection kit (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's instructions.

2.9. Statistical analyses

We determined the sample size for a pilot study using 2 independent-sample t tests and a power analysis. All data are presented as means $\pm$ standard error mean (SEM) of 3 separate experiments duplicated in each case. The difference between the control without PMACI and the control with PMACI was evaluated by an independent t test, and comparison among the PMACI-treated group and the cysteine-treated groups was analyzed by a 1-way analysis of variance (ANOVA) with a Tukey post hoc test using SPSS v12.00 statistical analysis software (SPSS Inc, Chicago, IL, USA). The results were deemed significant at a value of $P < .05$.

3. Results

3.1. Cysteine suppresses TSLP levels without inducing cytotoxicity

We initially investigated the inhibitory properties of nontoxic concentrations of cysteine on TSLP production outcomes using activated HMC-1 cells. First, an MTT assay was conducted to assess the cytotoxicity of cysteine. Various concentrations of cysteine (0.1, 1, and 10 μ g/mL) did not impact the viability of the cells (Fig. 1A). Fig. 1B showed that the incubation of HMC-1 cells with PMACI alone was associated with significantly greater production of TSLP compared with untreated cells, whereas cysteine (10 μ g/mL) with PMACI stimulation significantly inhibited TSLP production ($P < .05$). Cysteine (10 μ g/mL) without PMACI stimulation showed negligible TSLP production levels (Fig. 1B). Additionally, we sought to determine whether cysteine could regulate TSLP mRNA expression levels in PMACI-stimulated HMC-1 cells. TSLP mRNA expression outcomes were dramatically increased in cells with PMACI alone ($P < .05$); however, cysteine noticeably suppressed PMACI-induced TSLP mRNA expression levels (Fig. 1C and D, $P < .05$). Taken together, these data indicate that cysteine can suppress TSLP levels in activated mast cells.

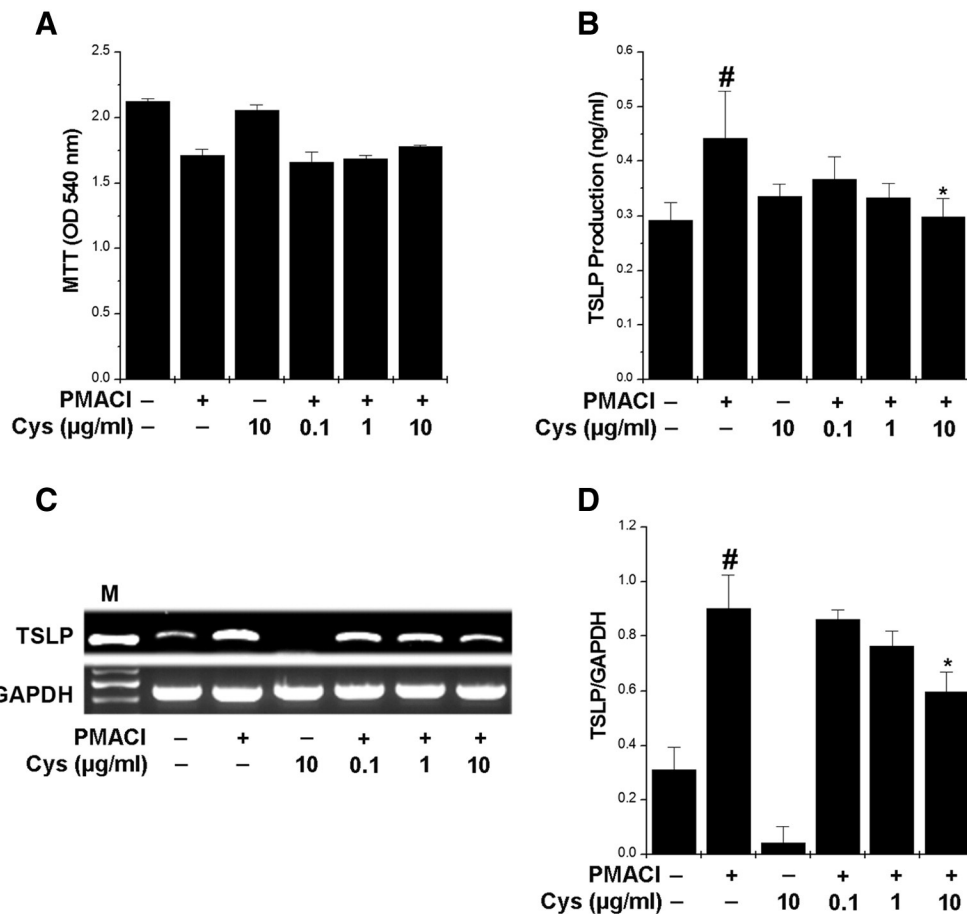


Fig. 1 – Effect of cysteine on the levels of TSLP without inducing cytotoxicity. **A**, MTT assay. **B**, HMC-1 cells (3×10^5) were treated with cysteine (0.1, 1, or 10 μg/mL) for 1 hour and were then stimulated by PMACI for 8 hours. The levels of TSLP in the supernatant were measured by the ELISA method. **C**, HMC-1 cells (1×10^6) were stimulated by PMACI. The level of mRNA was detected by the RT-PCR method. **D**, TSLP mRNA expression levels were quantitated by densitometry. Data represent the means $\pm$ SEM from 3 different experiments conducted in duplicate ($n = 6$). [#] $P < .01$; significantly different from unstimulated cells (independent t test). ^{*} $P < .05$; significantly different from PMACI-stimulated cells (1-way ANOVA with a Tukey post hoc test). PMACI, PMA plus A23187; Cys, cysteine; M, marker.

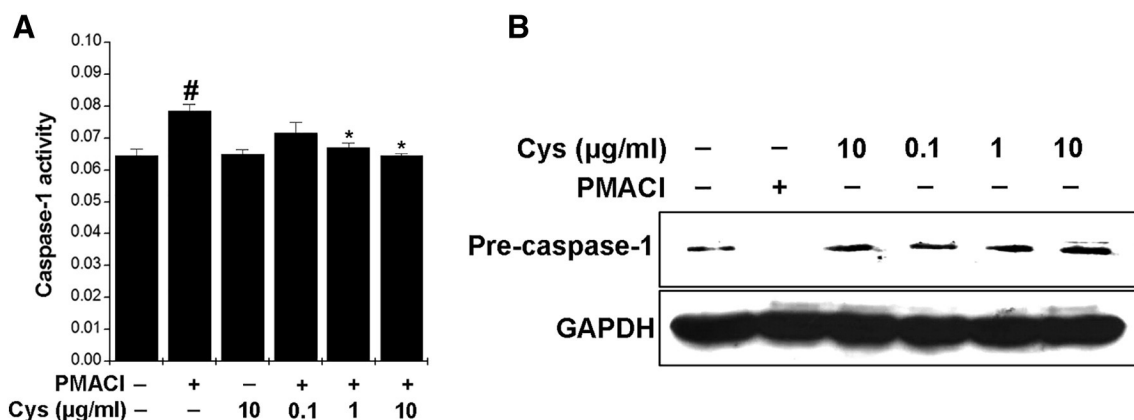


Fig. 2 – Effect of cysteine on the activation of caspase-1. HMC-1 cells (3×10^6) were treated with cysteine (0.1, 1, or 10 μg/mL) for 1 hour and then stimulated by PMACI for 2 hours. **A**, Caspase-1 activity levels were measured using a caspase-1 assay kit. Data represent the means $\pm$ SEM from 3 different experiments conducted in duplicate ($n = 6$). **B**, Expression levels of caspase-1 were analyzed by Western blotting. [#] $P < .01$; significantly different from unstimulated cells (independent t test). ^{*} $P < .05$; significantly different from PMACI-stimulated cells (1-way ANOVA with a Tukey post hoc test).

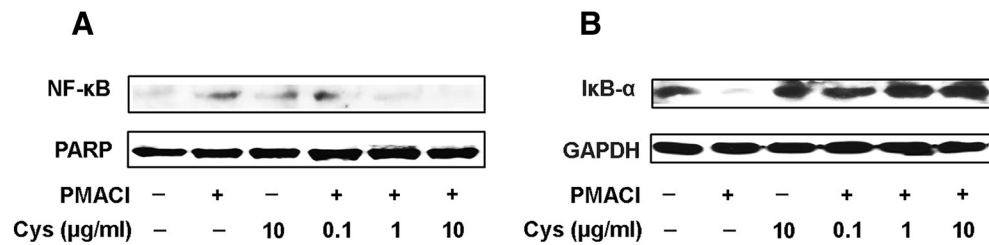


Fig. 3 – Effect of cysteine on the activation of NF-κB. HMC-1 cells (3×10^6) were treated with cysteine (0.1, 1, or 10 μg/mL) for 1 hour and then stimulated by PMACI for 2 hours. A, NF-κB (in nuclear extract) and (B) IκB-α (in cytosol extract) were determined by Western blotting.

3.2. Cysteine suppresses the activity of caspase-1

The caspase-1 pathway has been implicated in TSLP production. In addition, caspase-1 plays an indispensable role in AR immune reactions [17]. We estimated the properties of cysteine on caspase-1 activity in activated HMC-1 cells. As expected, stimulation with PMACI significantly increased caspase-1 activity in HMC-1 cells, but the activity of caspase-1 was significantly decreased in cells treated with cysteine (Fig. 2A, $P < .05$). Subsequently, the levels of caspase-1 pre-form were determined by Western blotting. The levels of pre-caspase-1 in PMACI-stimulated cells were strongly downregulated as

compared with those in untreated cells (Fig. 2B). Meanwhile, the downregulated pre-caspase-1 levels were increased by a treatment with cysteine (Fig. 2B). These results indicate that the inhibition of TSLP by cysteine may arise by suppressing caspase-1 activation.

3.3. Cysteine suppresses the translocation of NF-κB into the nucleus

NF-κB, a transcription factor, has been linked to TSLP mRNA expression. Therefore, suppressing the NF-κB pathway could be a potential therapeutic target. Therefore, we rule out

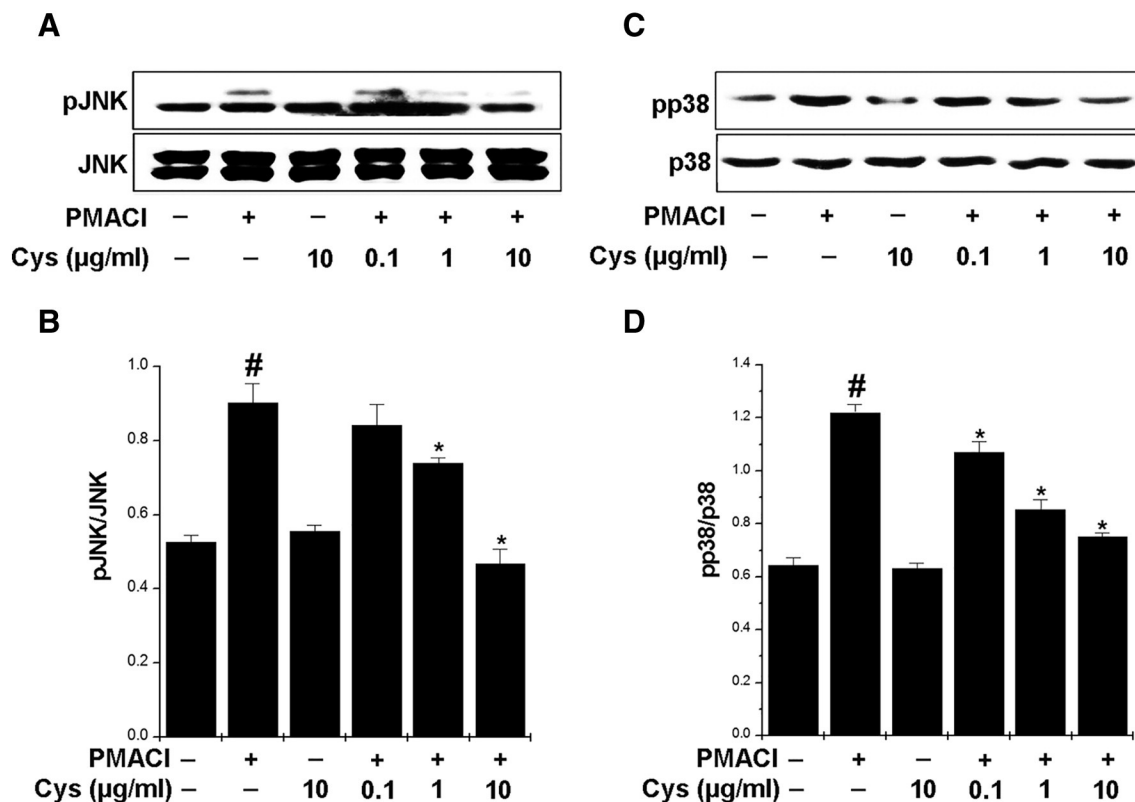


Fig. 4 – Effect of cysteine on the activation of MAPKs. HMC-1 cells (3×10^6) were treated with cysteine (0.1, 1, or 10 μg/mL) for 1 hour and then stimulated by PMACI for 30 minutes. A and C, Phosphorylated-JNK and p38 were determined by Western blotting. B and D, Relative densities were quantified by densitometry for pJNK/JNK and pp38/p38. Data represent the means $\pm$ SEM from 3 different experiments ($n = 3$). [#] $P < .01$; significantly different from unstimulated cells (independent t test). ^{*} $P < .05$; significantly different from PMACI-stimulated cells (1-way ANOVA with a Tukey post hoc test).

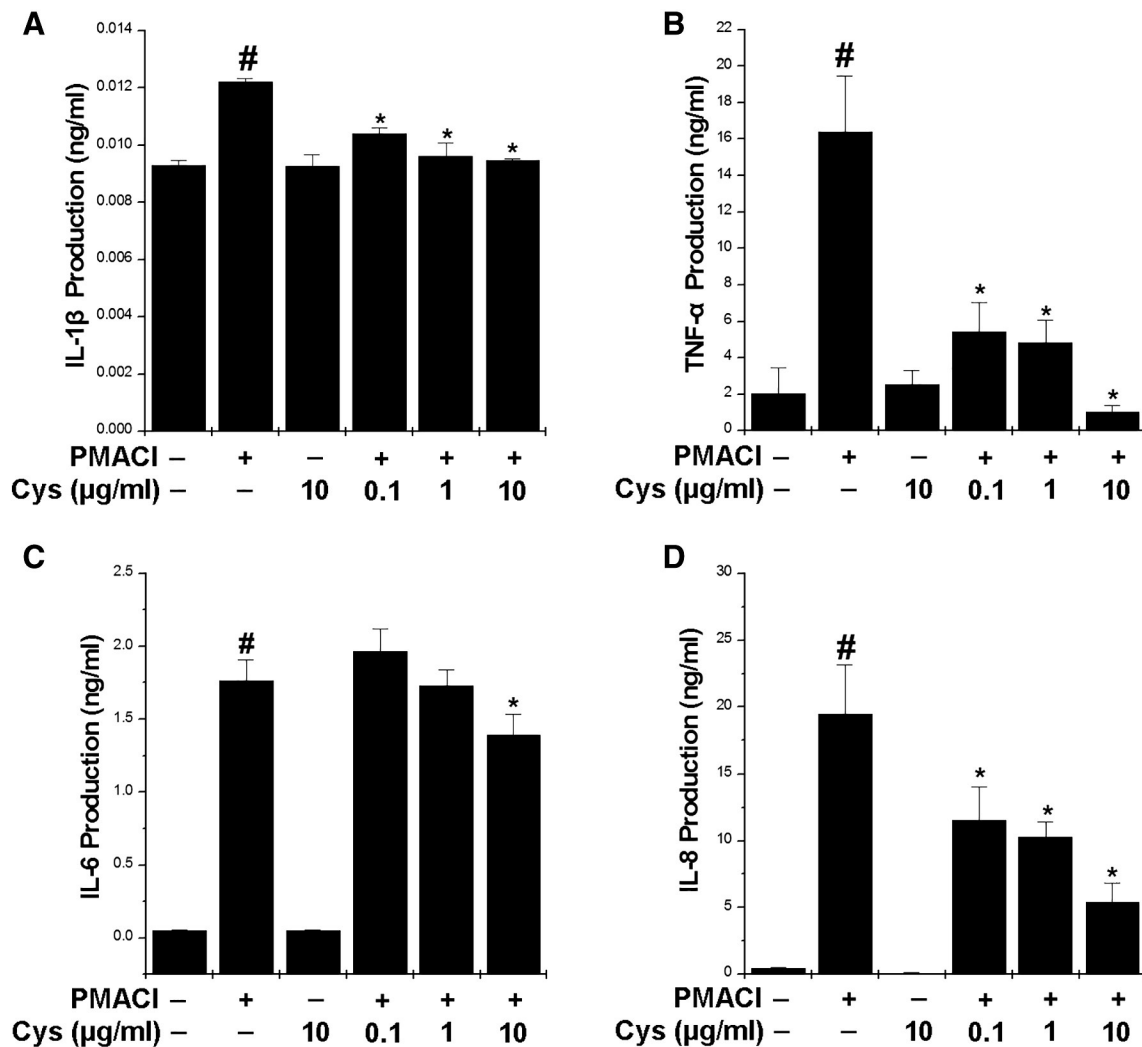


Fig. 5 – Effect of cysteine on the production of proinflammatory cytokines. HMC-1 cells (3×10^5) were treated with cysteine (0.1, 1, or 10 μ g/mL) for 1 hour and then stimulated by PMACI for 8 hours. A–D, Production of cytokines in the supernatant was measured by the ELISA method. Data represent the means $\pm$ SEM from three different experiments conducted in duplicate ($n = 6$). [#] $P < .01$; significantly different from unstimulated cells (independent t test). ^{*} $P < .05$; significantly different from PMACI-stimulated cells (1-way ANOVA with a Tukey post hoc test).

whether cysteine is able to affect the subsequent translocation of active NF- κ B into the nucleus. Here, we observed enhanced levels of NF- κ B in the nucleus of activated cells compared with unstimulated cells. Meanwhile, cysteine suppressed the translocation of NF- κ B from the cytoplasm into the nucleus at concentrations of 1 and 10 μ g/mL (Fig. 3A). Because NF- κ B translocation occurs following the degradation of I κ B- α in cytoplasm, the levels of I κ B- α in cytoplasm lysates were examined in the present study. As depicted in Fig. 3B, PMACI incubation led to strong downregulation of this signaling molecule (Fig. 3B). In contrast, this type of degradation of I κ B- α was inhibited by a pretreatment with cysteine (Fig. 3B). From this result, we can assume that the inhibitory properties of cysteine on TSLP mRNA expression were closely associated to the blocking of the NF- κ B-mediated pathway.

3.4. Cysteine suppresses the phosphorylation of mitogen-activated protein kinases

To estimate the mechanism by which cysteine inhibits TSLP production and mRNA expression levels, we investigated the activation of mitogen-activated protein kinase (MAPK) members (ERK, JNK, and p38). The activations of these members were analyzed by Western blotting. PMACI alone dramatically induced the phosphorylation of both JNK and p38 in HMC-1 cells, but these inductions were suppressed in a dose-dependent manner by a treatment with cysteine (Fig. 4A–D, $P < .05$). In particular, cells pretreated with cysteine at 10 μ g/mL reduced the phosphorylation of JNK and p38 to normal level (Fig. 4A–D, $P < .05$). Cysteine did not impact the phosphorylation of ERK (data not shown). This clearly suggests that cysteine inhibits TSLP production by blocking the JNK and p38 signal pathways.

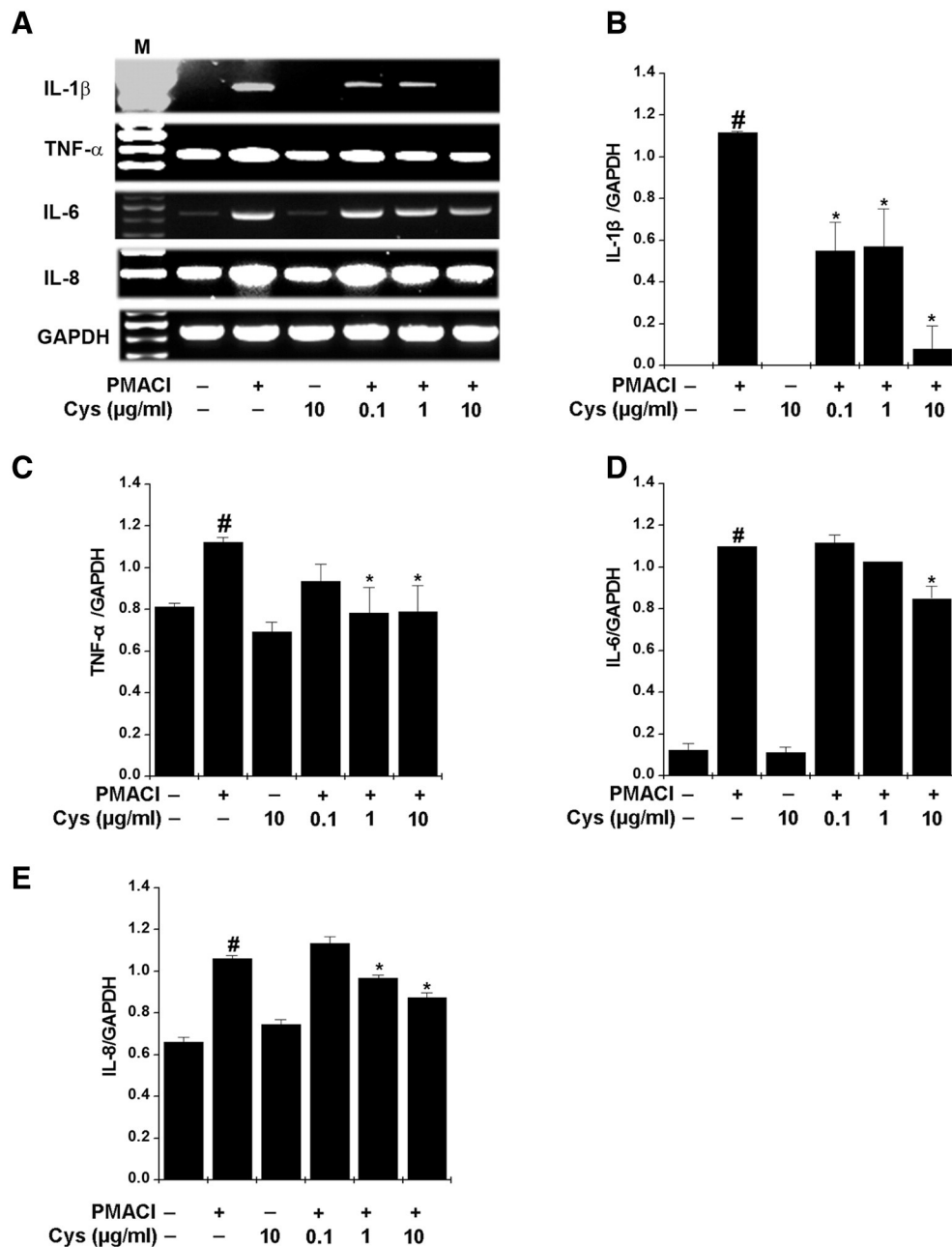


Fig. 6 – Effect of cysteine on the mRNA expression of proinflammatory cytokines. A, HMC-1 cells (3×10^6) were treated with cysteine (0.1, 1, or 10 $\mu\text{g/mL}$) for 1 hour and then stimulated by PMACI for 5 hours. IL-1 β , TNF- α , IL-6, and IL-8 mRNA expression levels were analyzed by RT-PCR. B, Relative intensities were quantified by densitometry for IL-1 β /GAPDH, TNF- α /GAPDH, IL-6/GAPDH, and IL-8/GAPDH. Data represent the means $\pm$ SEM from 3 different experiments with conducted in duplicate ($n = 6$). # $P < .01$; significantly different from unstimulated cells (independent t test). * $P < .05$; significantly different from PMACI-stimulated cells (1-way ANOVA with a Tukey post hoc test).

3.5. Cysteine suppresses the production and mRNA expression levels of IL-1 β , TNF- α , IL-6, and IL-8

The evidence showed that IL-8, TNF- α , IL-6, and IL-1 β are released predominantly by activated mast cells [18]. Therefore, the modulatory capabilities of cysteine on proinflammatory cytokine production were determined. PMACI alone induced a dramatic increase in the production of IL-1 β , TNF- α , IL-6, and IL-8, which were scarcely detectable in untreated cells (Fig. 5A-D). After a pretreatment with cysteine, the amounts of IL-1 β , TNF- α , IL-6,

and IL-8 proteins produced from PMACI-stimulated cells were significantly attenuated in a dose-dependent manner (Fig. 5A-D, $P < .05$). Cysteine (10 $\mu\text{g/mL}$) did not affect the amounts produced by itself (Fig. 5A-D). Finally, to examine whether cysteine could control proinflammatory cytokine transcriptional levels, mRNA expressions were detected by reverse transcription (RT)-PCR. PMACI resulted in apparent increases in IL-1 β , TNF- α , IL-6, and IL-8 mRNA expression by mast cells (Fig. 6A-E). Meanwhile, cysteine downregulated the expression levels of PMACI-induced IL-1 β , TNF- α , IL-6, and IL-8 mRNA (Fig. 6A-E, $P < .05$).

4. Discussion

This study showed that cysteine suppressed TSLP production from PMACI-stimulated HMC-1 cells by blocking caspase-1, NF- κ B, JNK, and p38. This finding suggests that cysteine is a candidate for the treatment of allergic inflammatory disorders.

Mast cells are critical participants in allergic diseases, such as asthma, atopic dermatitis, and AR [19]. It has been demonstrated that activated mast cells produce a wide spectrum of proinflammatory cytokines such as TSLP, IL-8, IL-1 β , IL-6, and TNF- α in response to multiple signals [20,21]. In particular, TSLP, a lymphocyte activating factor, is an extremely powerful molecule for immune reactions, and it initiates the Th2-type allergic inflammation because it promotes B-cell activation and immunoglobulin E production [22]. It is reported that IL-6, TNF- α , and IL-8 lead to the development of diverse inflammatory disorder and are upregulated in allergic inflammatory diseases [8,16,23]. It should also be noted that TSLP induced the production of these inflammatory cytokines and that it therefore can be regarded as a therapeutic molecule for allergic disorders. Gauvreau et al [24] reported that the anti-TSLP antibody inhibited eosinophilic inflammation and allergen-induced bronchoconstriction. In this study, we showed that cysteine markedly suppresses the production and mRNA expression levels of TSLP. In addition, cysteine clearly inhibited IL-1 β , TNF- α , IL-6, and IL-8 production in activated HMC-1 cells. These results indicate that cysteine can help to treat allergic inflammatory diseases.

The activation of caspase-1 mediates allergic inflammatory diseases and promotes the secretion of TSLP and proinflammatory cytokines [23,25,26]. The level of caspase-1 in an AR animal model was higher than that in normal control mice [27]. Yamanaka et al [28] showed that the overexpression of caspase-1 in mice increased serum histamine and immunoglobulin E levels. In addition, the levels of caspase-1 in patients with allergies are upregulated compared with those in healthy individuals [27]. Thus, the inhibition of caspase-1 is responsible for a reduced allergic inflammation levels. In the present study, cysteine markedly inhibited caspase-1 activation in PMACI-stimulated HMC-1 cells. Therefore, we suggest that caspase-1 can serve as a signaling molecule that is specifically targeted by cysteine.

Moreover, caspase-1 activates NF- κ B, and macrophages from caspase-1-deficient mice show reduced NF- κ B activity levels [25,29]. Several studies have proposed that TSLP at both mRNA and protein levels is modulated by NF- κ B in HMC-1 cells [25,30]. The activation of NF- κ B is controlled by I κ B α phosphorylation [31]. Recently, cysteine was found to inhibit TNF- α -induced NF- κ B activation in human coronary arterial endothelial cells [32]. In the present study, we observed that cysteine abrogated NF- κ B activation in HMC-1 cells. These findings indicate that the antiallergic inflammatory properties of cysteine can be mediated in part through the regulation of the NF- κ B pathway.

For a more thorough assessment of the inhibitory mechanism of cysteine on TSLP production, we determined the phosphorylation of MAPKs (ERK, JNK, p38) in PMACI-treated mast cells. Numerous studies have shown that the expression

levels of inflammatory cytokines are regulated by the activation of MAPKs [33,34]. The JNK cascade appears to be involved in stress reactions, whereas the p38 kinase is required in a variety of cytokine production steps. The suppression of p38 results in reduced IL-6 and TNF- α production levels [35]. The present study showed that cysteine inhibits PMACI-induced JNK and p38 phosphorylation levels, whereas this is not the case for ERK. Therefore, we assume that the antiallergic inflammatory properties of cysteine are mediated by control of the MAPK pathway.

In summary, our results support the hypothesis that cysteine has an antiallergic inflammatory effect by inhibiting TSLP production by blocking the caspase-1/NF- κ B pathways in HMC-1 cells. Blocking of mast cell-derived inflammatory mediators may be beneficial for the prevention or treatment of allergic inflammatory conditions. Overall, this study provides evidence that cysteine is likely an important factor in the development of functional foods or therapeutic drugs to prevent or treat allergic inflammatory disorders. However, further studies are needed to elucidate the antiallergic inflammatory effects of cysteine in animal models and clinical trials.

Acknowledgment

HJJ and HMK designed the research; SYN conducted the research, performed statistical analysis, and wrote the manuscript. HJJ and HMK have primary responsibility for final content. All authors have no conflicts of interest. This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2015R1A1A3A04000922). The authors declare no conflict of interest.

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