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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–κB 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–κB, p38, and c-Jun N-terminal kinase–dependent pathways in activated HMC-1 cells, suggesting its potential as a regulator of allergic inflammatory diseases.

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, bicinchoninic 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-κB, nuclear factor–κB; 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)-1β, 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-1β 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-acetate4 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)–κB, 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-1β/TNF-α/IL-8 antibodies (Abs), biotinylated anti-human TSLP/IL-6/IL-1β/TNF-α/IL-8 Abs, and recombinant human TSLP/IL-6/IL-1β/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-κB, 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% CO_2 and 95% humidity in Isocove's modified Dulbecco's medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 10 μmol/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 μmol/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 μg/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 μg 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-1β, 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.