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Short Communication

Thymic stromal lymphopoietin is expressed and produced by caspase-1/NF-κB pathway in mast cells

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ABSTRACT

Thymic stromal lymphopoietin (TSLP) plays a pivotal role in allergic diseases such as atopic dermatitis, asthma, and chronic obstructive pulmonary disease. Although there are many reports regarding function and regulatory mechanism of TSLP in dendritic cells and/or T cells, the regulatory mechanism of TSLP in mast cells has not been fully elucidated. Here, we describe how TSLP is expressed and produced by inflammatory stimulus in mast cells. TSLP mRNA was expressed by phorbol myristate acetate (PMA) plus A23187 stimulation in HMC-1 cells and reached its peak 5 h after PMA plus A23187 stimulation. The expression of TSLP mRNA was inhibited by nuclear factor (NF)-κB inhibitor. In addition, NF-κB luciferase activity was inhibited by caspase-1 inhibitor, indicating that caspase-1 is an upstream of NF-κB in mast cells. Furthermore, caspase-1 inhibitor decreased the expression of TSLP mRNA induced by PMA plus A23187. Finally, TSLP production was inhibited by both caspase-1 inhibitor and NF-κB inhibitor. These results provide proof of principle that TSLP can be expressed and produced through caspase-1 and NF-κB in mast cells and open new perspectives to pharmacologically manipulate the expression and production of TSLP by molecules acting on the caspase-1 and NF-κB pathway.

1. Introduction

Atopic dermatitis (AD) is a common skin disease that is often associated with other atopic disorders, such as asthma and allergic rhinitis. AD, characterized by pruritic and eczematoid skin lesions, affects up to 20% of children and 1–3% of adults in most countries of the world [1,2]. The most common form of AD, accounting for 70–80% of cases, is allergic AD with elevated concentrations of total and allergen-specific immunoglobulin (Ig)E in serum and skin. The remaining 20–30% patients have nonallergic AD, with normal total IgE levels and negative serum allergen-specific IgE, reflecting multiple different pathogenic mechanisms and indicating that AD represents a collection of heterogeneous groups [3]. As a result of the increasing prevalence of atopic disorders in developed countries, the burden of healthcare cost increases and the quality of life of affected patients is significantly lowered by chronic eczematous lesions, pruritus, sleep loss, dietary restrictions and psychosocial affections [2].

Thymic stromal lymphopoietin (TSLP) was originally identified as a growth factor in culture supernatants of a thymic stromal cell line to support the development of murine B cells in 1994 [4]. In humans, TSLP is an IL-7-like cytokine molecule that was first cloned in 2001 [5]. TSLP plays a pivotal role in allergic diseases such as AD, asthma, and chronic obstructive pulmonary disease [6]. Initially, TSLP was found to enhance potently the maturation of CD11c$^+$ dendritic cells, and TSLP-primed and activated dendritic cells promoted differentiation of naive CD4$^+$ T cells into proinflammatory T_H2 cells [7]. High expression of TSLP is a feature of keratinocytes in AD skin lesions, and TSLP-priming of dendritic cells *in situ* may serve to induce or enhance T_H2 responses within the skin, as well as systemically. Consistent with this viewpoint, TSLP was originally reported to exert its T_H2-promoting properties through a DC-mediated pathway in human beings that involved induction of OX40 ligand on dendritic cells [8]. Even though there are many reports regarding function and regulatory mechanism of TSLP in dendritic cells and/or T cells, the regulatory mechanism of TSLP in mast cells has not been fully elucidated. In fact, almost all of reports that had investigated for TSLP in mast cells showed only the results using TSLP as a stimulant.

In atopic diseases such as asthma, AD, and allergic rhinitis, not only epithelial cells, DCs, eosinophils, and T cells but also mast cells are important. A number of studies reported that mast cells are activated and infiltrated in the skin lesion of AD animal model, suggesting the contribution of mast cells in AD [9–14]. Thus, we investigated the regulatory mechanism of TSLP in mast cells.

Caspase-1 is a member of the caspase family [15]. Quite unlike the role that most caspases have in apoptosis, caspase-1 mainly

Abbreviations: AD, atopic dermatitis; NF-κB, nuclear factor-κB; PMA, phorbol myristate acetate; PDTC, pyrrolidine dithiocarbamate.

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serves to cleave IL-1β and IL-18 from their inactive precursors to their active forms [16,17]. In addition to the well-established role of caspase 1 in the maturation of IL-1β and IL-18, caspase 1 is also capable of activating the nuclear factor (NF)-κB [18]. Activated NF-κB mediates induction of TSLP gene expression in airway epithelial cells [19]. Here, we report that TSLP is expressed and produced through caspase-1/NF-κB pathway in mast cells.

2. Materials and methods

2.1. Reagents

Phorbol myristate acetate (PMA) and A23187 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). We purchased IMDM from Gibco BRL (Grand Island, NY, USA); TMB substrate from Pharmingen (Sandiego, CA, USA); caspase-1 inhibitor from R&D Systems (Minneapolis, MN, USA).

2.2. Cell culture

Human mast cell line, HMC-1 cells were grown in IMDM and supplemented with 100 Units/ml of penicillin, 100 μg/ml of streptomycin, and 10% fetal bovine serum at 37 °C in 5% CO_2 with 95% humidity. We stimulated the cells with 0.05 μM of PMA plus 1 μM of A23187 and incubated them at 37 °C for indicated time.

2.3. Cytokine assay

We used enzyme-linked immunosorbent assay (ELISA) method to assay the culture supernatant for TSLP [20]. Sandwich ELISA for TSLP was carried out in duplicate in 96 well ELISA plate. First, we coated plate with 100 μl aliquots of mouse anti-human TSLP monoclonal antibody at 1.0 μg/ml in PBS at pH 7.4 and incubated plate overnight at 4 °C. The plate was washed in PBS containing 0.05% Tween-20 (Sigma) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 for 1 h. After additional washes, the culture supernatant and TSLP standards were added and incubated at 37 °C for 2 h. After 2 h incubation at 37 °C, the wells were washed and then each of 0.2 μg/ml of biotinylated anti-human TSLP was added and again incubated at 37 °C for 2 h. After washing the wells, streptavidin-peroxidase was added and plate was incubated for 20 min at 37 °C. Wells were again washed and TMB substrate (Pharmingen) was added. Color development was measured at 450 nm using an automated microplate ELISA reader. A standard curve was run on plate using recombinant human TSLP in serial dilutions.

2.4. Reverse transcription–polymerase chain reaction (RT–PCR) analysis

We used the method of Moon et al. [20] using an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Republic of Korea) and isolated the total RNA from HMC-1 cells in accordance with the manufacturer's specification. The concentration of total RNA in the final elutes was determined by spectrophotometer. Total RNA (1 μg) 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 cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). PCR was performed with following primer for human TSLP (5′ TAT GAG TGG GAC CAA AAG TAC CG 3′; 5′ GGG ATT GAA GGT TAG GCT CTG G 3′). GAPDH (5′ CAA AAG GGT CAT CAT CTC TG 3′; 5′ CCT GCT TCA CCA CCT TCT TG 3′) was used to verify if equal amounts of RNA were used for reverse transcription and PCR amplification from different experimental conditions. The annealing temperature was 62 °C for TSLP and GAPDH. Amplified fragment sizes for TSLP and GAPDH were 97 and 446 bp, respectively. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

2.5. Transient transfection and luciferase assay

For the transfection, we seeded HMC-1 cells (1×10^7) in a 100 mm culture dish. We then used Lipofectamine™2000 purchased from Invitrogen (Carlsbad, CA, USA) to transiently transfect pNF-κB luciferase (LUC) and pSV40-LUC reporter gene constructs into HMC-1 cells. To measure the luciferase activity, we used a luminometer 1420 luminescence counter purchased from Perkin Elmer (Waltham, MA, USA) in accordance with the manufacturer's protocol. All the transfection experiments were performed in at least three different experiments, with similar results. The luciferase activity was defined as the ratio of *firefly* luciferase activity to *renilla* luciferase activity.

2.6. Statistical analysis

All results are expressed as the mean ± SEM. The statistical evaluation of the results was performed by an independent *t*-test and an ANOVA with a Tukey post hoc test. The results were significant with a value of $P < 0.05$.

3. Results and discussion

3.1. mRNA expression of human TSLP in HMC-1 cells

It was reported that immunostimulant poly I:C induced TSLP mRNA expression in human conjunctival epithelial cells [21]. Thus, we investigated whether the mRNA of human TSLP is expressed by inflammatory stimulus in human mast cell line, HMC-1 cells. When we stimulated the HMC-1 cells with PMA plus A23187 (Calcimycin; $C_{29}H_{37}N_3O_6$), the mRNA of TSLP was expressed (Fig. 1A). In the present study, we used protein kinase C activator PMA as a substitute for diacylglycerol and A23187 for ionophore. Furthermore, we examined when the mRNA expression of TSLP reaches its peak in HMC-1 cells. The expression of TSLP mRNA was reached its peak 5 h after PMA plus A23187 stimulation (Fig. 1B). As time went on, the mRNA expression of IL-6 was also increased when we stimulated the HMC-1 cells with PMA plus A23187 (Fig. 1C). As shown in Fig. 1C and D, the mRNA of IL-6 was expressed in unstimulated cells from 2 h after PMA plus A23187 stimulation, and spontaneous mRNA expression of IL-6 persist until the end of experiment. However, the expression of TSLP mRNA was induced by only PMA plus A23187 stimulation. Therefore, we assume that TSLP is a specialist that is in charge of only signaling an inflammation. Because the TSLP mRNA was seldom expressed by any other factors except for PMA plus A23187 stimulation.

3.2. Inhibition of human TSLP mRNA expression by NF-B inhibitor, PDTC

A number of studies reported that the expression of human TSLP mRNA was controlled by NF-κB in various cells such as fibroblasts and epithelial cells [19,22,23]. Our result also showed that the mRNA expression of TSLP was inhibited by NF-κB inhibitor (pyrrolidine dithiocarbamate, PDTC; 10 and 100 μM; Fig. 2A). Thus, we confirmed that NF-κB is a general transcription factor in fibroblasts and epithelial cells as well as mast cells.