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SHORT COMMUNICATION

The suppression of thymic stromal lymphopoietin expression by selenium

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Abstract Thymic stromal lymphopoietin (TSLP) is a key mediator of allergic diseases such as allergic rhinitis, asthma, and atopic dermatitis. Selenium (Se) has various effects such as antioxidant, antitumor, antiulcer, and anti-inflammatory effects. However, the effect of Se on the production of TSLP has not been clarified. Thus, we investigated how Se inhibits the production of TSLP in the human mast cell line, HMC-1 cells. Se suppressed the production and mRNA expression of TSLP in HMC-1 cells. The maximal inhibition rate of TSLP production by Se (10 μM) was 59.14 ± 1.10%. In addition, Se suppressed the nuclear factor-κB luciferase activity induced by phorbol myristate acetate plus A23187. In the activated HMC-1 cells, the activation of caspase-1 was increased; whereas the activation of caspase-1 was decreased by pretreatment with Se. These results suggest that Se can be used to treat inflammatory and atopic diseases through the suppression of TSLP.

Keywords Thymic stromal lymphopoietin · Selenium · Nuclear factor-κB · Caspase-1

Abbreviations

AD	Atopic dermatitis
Se	Selenium
NF-κB	Nuclear factor-κB
PMA	Phorbol myristate acetate
TSLP	Thymic stromal lymphopoietin

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Introduction

Atopic dermatitis (AD) is a chronic, recurring, pruritic, inflammatory skin condition which has its onset in early childhood in most individuals and has an important impact on life quality (Blume-Peytavi and Wahn 2011). The lifetime prevalence of AD is estimated to 15–30% in children and 2–10% in adults, while the incidence of AD has increased by 2- to 3-fold during the past 3 decades in industrialized countries (Bieber 2010). Thus AD has, and will continue to have, significant socioeconomic and personal impacts in these countries (Mancini et al. 2008).

Thymic stromal lymphopoietin (TSLP) was found to enhance potently the maturation of $CD11c^+$ dendritic cells, and TSLP-primed and activated dendritic cells promoted the differentiation of naive $CD4^+$ T cells into pro-inflammatory T_H2 cells (Liu 2006). A 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 dendritic cell-mediated pathway in human beings that involved the induction of the OX40 ligand on dendritic cells (Reefer et al. 2010). TSLP has been implicated in the development of asthma and AD (Kashyap et al. 2011). In atopic diseases such as asthma and AD, not only dendritic cells, epithelial cells, 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 the AD animal model, suggesting the contribution of mast cells in AD (Oiwa et al. 2008; Schneider et al. 2009; Dumortier et al. 2010; Hong et al. 2011).

Cysteine protease caspase-1 is a member of the caspase family (Gordon et al. 1990). Quite unlike the roles that

most caspases have in apoptosis, caspase-1 mainly serves to cleave IL-1β and IL-18 from their inactive precursors to their active forms (Boost et al. 2007; Lamkanfi et al. 2007). In addition to the well-established roles of caspase 1 in the maturation of IL-1β and IL-18, caspase 1 is also capable of activating the nuclear factor (NF)-κB (Lamkanfi et al. 2004). The activated caspase-1 activates NF-κB in HMC-1 cells (Moon and Kim 2011). NF-κB activated by caspase-1 mediates the induction of TSLP gene expression in airway epithelial cells (Lee and Ziegler 2007).

Selenium (Se) is an essential nutritional trace element involved in different physiological functions with antioxidant, antitumor, antiulcer, and anti-inflammatory properties (Rayman 2000; Duntas 2009; Bhattacharya et al. 2011; Kim et al. 2011). However, the effect of Se on the production of TSLP has not yet been clarified. Thus, we investigated how Se suppresses the production of TSLP in mast cells.

Materials and methods

Reagents

Phorbol myristate acetate (PMA), A23187, and Se were purchased from Sigma Chemical Co. (St. Louis, MO, USA). We purchased IMDM from Gibco BRL (Grand Island, NY, USA); TSLP antibodies from R&D Systems (Minneapolis, MN, USA); TMB substrate from Pharmingen (San Diego, CA, USA); RIP2 and caspase-1 antibodies from Santa Cruz Biotechnology (Santa Cruz, CA, USA); GAPDH antibody from Assay Designs Inc. (Ann arbor, MI, USA).

Cell culture

The 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.

Cytokine assay

We used the enzyme-linked immunosorbent assay (ELISA) method to assay the culture supernatant for TSLP (Moon et al. 2011). A sandwich ELISA for TSLP was carried out in duplicate in a 96-well ELISA plate. First, we coated the 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 the 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 the 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 the plate was incubated for 20 min at 37°C. The 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 the plate using recombinant human TSLP in serial dilutions.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

We used the method of Moon et al. (2011), 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 specifications. The concentrations of total RNA in the final elutes were determined by a 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 a cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The PCR was performed with the following primers for human TSLP (5′TAT GAG TGG GAC CAA AAG TAC CG3′; 5′GGG ATT GAA GGT TAG GCT CTG G3′). GAPDH (5′CAA AAG GGT CAT CAT CTC TG3′; 5′CCT GCT TCA CCA CCT TCT TG3′) was used to verify if equal amounts of RNA were used for reverse transcription and PCR amplification under 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.

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 relative luciferase activity was defined as the ratio of *firefly* luciferase activity to *renilla* luciferase activity.

Preparation of nuclear extracts

Nuclear extracts were prepared as described previously (Schoonbroodt et al. 1997). Briefly, after activating the

cells for the times indicated, we washed 5×10^6 cells in ice-cold PBS and centrifuged them at 15,000*g* for 1 min. We then resuspended them in 40 μl of a cold hypotonic buffer (10 mM Hepes/KOH, 2 mM $MgCl_2$, 0.1 mM EDTA, 10 mM KCl, 1 mM DTT, and 0.5 mM PMSF, pH 7.9). Next, we allowed the cells to swell on ice for 15 min; we lysed them gently with 2.5 μl of 10% Nonide P (NP)-40. We then centrifuged them at 15,000*g* for 3 min at 4°C. The supernatant was discarded and the pellets were gently resuspended in 40 μl of cold saline buffer (50 mM HEPES/KOH, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, and 0.5 mM PMSF, pH 7.9) and left on ice for 20 min. After conducting the centrifugation (15,000*g* for 15 min at 4°C), we froze the aliquots of supernatant containing the nuclear proteins in liquid nitrogen and stored them at −70°C until ready for analysis. Finally, we used the bicinchoninic acid protein assay (Sigma, St. Louis, MO, USA) to measure the protein concentrations.

Western blot analysis

The cell lysates were prepared in a sample buffer containing sodium dodecyl sulfate (SDS). The samples were heated at 95°C for 5 min and briefly cooled on ice. Following the centrifugation at 15,000*g* for 5 min, the proteins in the cell lysates were then separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose paper. The membrane was then blocked with 5% skim milk in PBS–tween-20 for 1 h at room temperature and then incubated with the primary and secondary antibodies. Finally, the protein bands were visualized by an enhanced chemiluminescence assay purchased from Amersham Co. (Newark, NJ, USA) following the manufacturer's instructions.

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 $P < 0.05$.

Results

Effect of Se on the production of TSLP in HMC-1 cells

To investigate the inhibitory effect of Se on the production of TSLP, we stimulated HMC-1 cells with PMA plus A23187 for 7 h, and we used the ELISA to analyze the supernatants for TSLP. The stimulation with PMA plus A23187 increased TSLP production from HMC-1 cells (Fig. 1a). The levels of TSLP that increased due to PMA plus A23187 were significantly decreased by Se (10 μM) (Fig. 1a, $P < 0.05$). The maximal inhibition rate of TSLP production by Se (10 μM) was 59.14 ± 1.10%. When Se was given as a pretreatment at various concentrations ranging from 0.1 to 10 μM, the cytotoxicity by Se was not shown (data not shown).

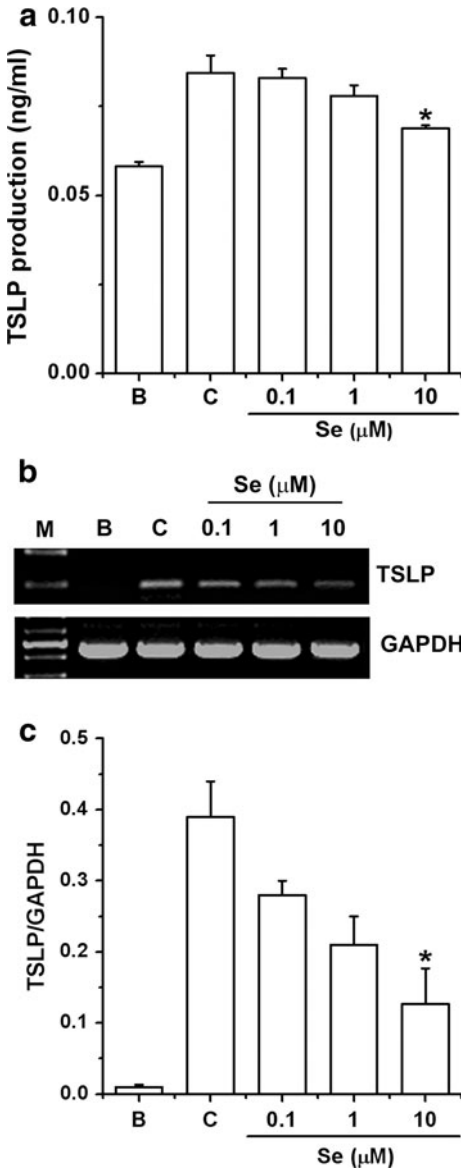

Fig. 1 Effects of Se on the production and mRNA expression of TSLP in HMC-1 cells. **a** HMC-1 cells (4×10^5) were treated with various concentrations (0.1 to 10 μM) of Se for 2 h, and then stimulated with PMA plus A23187 for 7 h. The levels of TSLP in the supernatant were measured with the ELISA method. **b** HMC-1 cells (1×10^6) were stimulated with PMA plus A23187. The mRNA was measured with the RT-PCR method. **c** The TSLP mRNA expression levels were quantified by densitometry. *M* marker, *B* unstimulated cells, *C* vehicle-treated, and then PMA plus A23187-stimulated cells

Effect of Se on the mRNA expression of TSLP in HMC-1 cells

To examine whether Se can modulate PMA plus A23187-induced mRNA expression of TSLP, we pretreated the cells with Se for 2 h before the PMA plus A23187 stimulation. We stimulated the cells with PMA plus A23187 for 5 h and then performed the RT-PCR analysis. The mRNA expression of TSLP was up-regulated by PMA plus A23187, but the up-regulated TSLP mRNA expression was decreased by the treatment with Se (Fig. 1b). The inhibitory effect of 10 μM of Se was greater than 0.1 and 1 μM, thus we evaluated the effect of 10 μM of Se in the next set of experiments: luciferase assay and Western blot analysis.

Effect of Se on the activation of NF-κB in HMC-1 cells

To determine whether Se could modulate the luciferase expression specifically via NF-κB activation, we performed a dual-luciferase assay. As shown in Fig. 2, the PMA plus A23187 stimulation increased the reporter gene activity. However, the increased NF-κB luciferase activity was significantly decreased by Se (10 μM, $P < 0.05$). The relative luciferase activity at the dose of 10 μM was 20.87 ± 1.20. The control and spontaneous values were 33.29 ± 1.73 and 0.94 ± 0.09, respectively.

Effect of Se on the activation of caspase-1 in HMC-1 cells

Finally, to examine the effect of Se on the activation of caspase-1, we performed a Western blot analysis for caspase-1 in cells stimulated by PMA plus A23187. In the control group that was stimulated by PMA plus A23187, the levels of active caspase-1 were increased in HMC-1 cells. However, the levels of active caspase-1 were decreased by pretreatment with Se (Fig. 3).

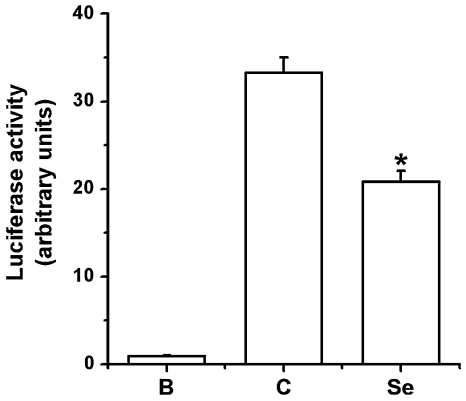

Fig. 2 Effects of Se on the activation of NF-κB in HMC-1 cells. HMC-1 cells (1×10^7) were transiently transfected pNF-κB-LUC and pSV40-LUC into and treated with Se (10 μM) for 2 h, and then stimulated with PMA plus A23187 for 48 h. The NF-κB activity was assessed with a luciferase assay. *B* unstimulated cells, *C* vehicle-treated, and then PMA plus A23187-stimulated cells; *Se* 10 μM of Se-treated, and then PMA plus A23187-stimulated cells. Each datum represents the mean ± SEM of three independent experiments. $*P < 0.05$, significantly different from the control (vehicle-treated, and then PMA plus A23187-stimulated cells) value

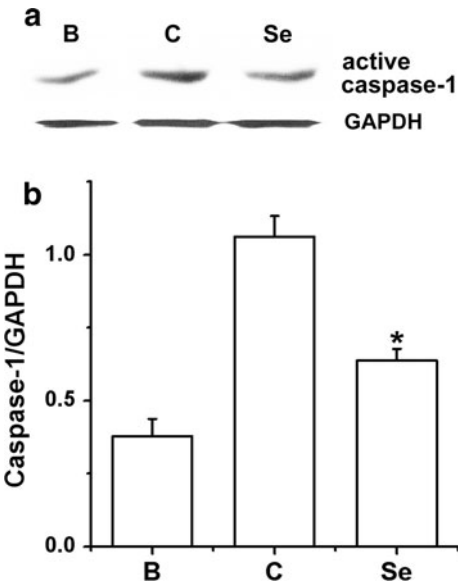

Fig. 3 Effects of Se on the activation of caspase-1 in HMC-1 cells. **a** HMC-1 cells (5×10^6) were preincubated for 2 h with Se (10 μM) and stimulated with PMA plus A23187 for 1 h. The total proteins were determined by Western blot analysis. **b** Each protein level was quantified by densitometry. *B* unstimulated cells, *C* vehicle-treated, and then PMA plus A23187-stimulated cells, *Se* 10 μM of Se-treated, and then PMA plus A23187-stimulated cells. Each datum represents the mean ± SEM of three independent experiments. $*P < 0.05$, significantly different from the control (vehicle-treated, and then PMA plus A23187-stimulated cells) value

Discussion

Chemical elements such as selenium, fluoride, iron, calcium and magnesium are essential to human beings even though some are toxic when absorbed in high doses (Gore et al. 2010). Among them, Se has various beneficial effects such as antioxidant, antitumor, antiulcer, and anti-inflammatory effects (Rayman 2000; Duntas 2009; Bhattacharya et al. 2011; Kim et al. 2011). Furthermore, Se-enriched probiotics have been shown to strongly inhibit the growth of pathogenic *Escherichia coli* in vivo and in vitro (Yang et al. 2009). Therefore, we hypothesized that Se can help us to treat inflammation and AD through the inhibition of TSLP production.

The PKC activator PMA is generally a substitute for diacylglycerol, and A23187 is a widely used ionophore. Our previous study showed that TSLP is produced by PMA plus A23187 stimulation (Moon and Kim 2011). Lesional, but not unaffected, skin from patients with AD expressed

high levels of TSLP (Ziegler 2010). In the present study, Se suppressed the production and mRNA expression of TSLP (Fig. 1). To our knowledge, this is the first study showing an inhibition of TSLP by Se in mast cells. Thus, we presume that Se might have significant potential in the treatment of inflammation and AD.

It has been reported that the expression of human TSLP mRNA was controlled by NF-κB in various cells such as fibroblasts and epithelial cells (Lee and Ziegler 2007; Ozawa et al. 2007; Lee et al. 2008). The expression and production of TSLP was controlled by NF-κB in mast cells (Moon and Kim 2011). Youn et al. (2008) reported that Se suppresses LPS-induced NF-κB activation in RAW 264.7 cells. In addition, Se inhibited LPS-induced nuclear translocation of NF-κB in murine macrophage cells, J774A.1 cells (Kim et al. 2004). Our results also showed that Se inhibited the NF-κB luciferase activity in mast cells (Fig. 2). From previous reports and our results, we could confirm that NF-κB is a general transcription factor in fibroblasts, epithelial cells, macrophages, and mast cells.

Upon receipt of a pro-inflammatory stimulus, caspase-1 is activated (Humke et al. 2000). Our results also showed that caspase-1 was activated by pro-inflammatory stimulus in HMC-1 cells (Fig. 3). Pretreatment of Se inhibited PMA plus A23187-induced activation of caspase-1 (Fig. 3). To our knowledge, this is also the first study showing an inhibition of caspase-1 activation by Se in mast cells. Thus, we can assume that Se inhibits the expression and production of TSLP through the blocking of caspase-1 in mast cells.

In conclusion, we have shown that Se can regulate the inflammatory responses induced by PMA plus A23187 in mast cells. Se suppressed the expression and production of TSLP through the blocking of caspase-1 and NF-κB pathways. Overall, this study, summarized in Fig. 4, suggests that Se has a potential in the treatment of inflammatory and atopic diseases through the suppression of TSLP.

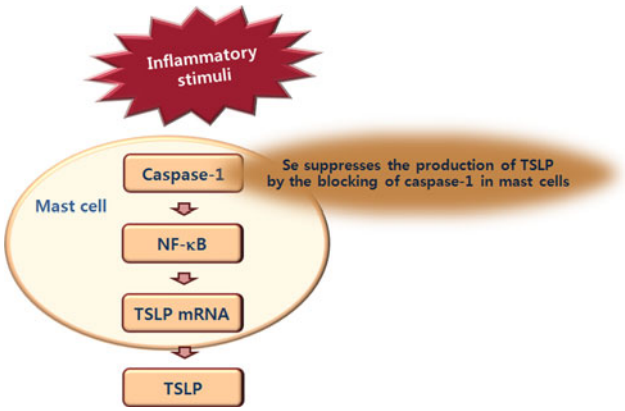

Fig. 4 Schematic diagram of proposed regulation of TSLP by Se. Upon receipt of pro-inflammatory stimuli, caspase-1 is activated, and then the activated caspase-1 activates NF-κB in mast cells. Finally, the activated NF-κB induces the expression of TSLP mRNA, followed by the production of TSLP in mast cells. In this study, Se suppressed the production of TSLP through the blocking of caspase-1 in mast cells

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Conflict of interest The authors declare that they have no conflict of interest.

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