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Loss of SOCS7 in mice results in severe cutaneous disease and increased mast cell activation

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Abstract The Suppressor of Cytokine Signaling (SOCS) protein family plays a central role in the negative regulation of cytokine action and has been implicated in the development of atopic diseases. Lack of SOCS7 is associated with severe skin disease in mice. We sought to explore the underlying mechanisms resulting in this phenotype. Skin samples were analyzed and serum immunoglobulin production was measured. Cytokine production by bone marrow derived mast cells was determined by ELISA. Mast cell thymic stromal lymphopoietin (TSLP) production was assessed by quantitative real-time PCR. Data obtained revealed that *Socs7*$^{-/-}$ mice have increased serum IgE and IgG_1 production and exhibit an increased mast cell infiltrate, as well as un-provoked mast cell degranulation in the dermis as compared to controls. *In vitro*, bone marrow derived mast cells from *Socs7*$^{-/-}$ mice are hyperactive to IgE-mediated stimuli, with elevated production of pro-inflammatory cytokines (IL-13, IL-6, TNF-α). Further, activated *Socs7*$^{-/-}$ bone marrow derived mast cells have increased IL-7Rα transcript, which is part of the heterodimeric receptor for TSLP. Finally, lack of SOCS7 was accompanied by an increase in TSLP mRNA and protein production by mast cells following FcεRI aggregation. These data implicate SOCS7 in the modulation of allergic inflammation and demonstrate that SOCS7 is involved in the regulation of TSLP signaling in mast cells.

Introduction

The Suppressors of Cytokine Signaling (SOCS) family proteins have been associated with inflammatory diseases such as psoriasis and atopic dermatitis [1,2]. The SOCS protein family consists of eight members, CIS1 and SOCS1 through 7. These family members share similar structural motifs including a central SH2 domain and a SOCS box at the C

Abbreviations: AD, Atopic Dermatitis; SOCS, Suppressor of Cytokine Signaling; TSLP, Thymic Stromal Lymphopoietin; TSLPR, TSLP Receptor; BMMC, Bone Marrow Derived Mast Cells; DNP-HSA, 2,4-Dinitrophenol-Human Serum Albumin; Ig, Immunoglobulin.

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terminus. SOCS proteins are regulated by a variety of cytokines and function in a classical negative feedback loop of cytokine signaling [3].

Mast cells which originate from pluripotential precursors and are critical effector cells in allergic inflammatory diseases, may be influenced by members of the SOCS family [4]. Inhibition of mast cell development induced by IFN-γ has thus been shown to involve SOCS1 [5–7]. Similarly, bone marrow obtained from *Socs1*$^{-/-}$ mice fails to give rise to viable mast cells following culture in IL-3 and stem cell factor [7]. These *Socs1*$^{-/-}$ mice also die perinatally of a Th1 driven inflammatory disease which results in multiple organ failure that is accompanied by a significant decrease in peritoneal mast cells [7,8].

SOCS7 is one of the least studied members of the SOCS family, and has been reported to have a role in insulin-initiated signaling [9] and to be part of a signaling pathway which is linked to DNA damage checkpoint responses [10]. In a 129/Bl6 mixed background, approximately half of *Socs7*$^{-/-}$ mice have been observed to develop severe dermatitis. This finding, along with a role for SOCS1 in mast cell survival, led us to enter into experiments to determine how the absence of SOCS7 may promote dermatitis.

As will be shown, histopathology from sites of dermatitis in *Socs7*$^{-/-}$ mice revealed an increase in the number of skin mast cells; and that these mast cells appeared to be undergoing degranulation. This finding was associated with an increase in serum IgG1 and IgE levels. Mast cells derived from bone marrow of *Socs7*$^{-/-}$ mice displayed an exaggerated cytokine release profile following FcεRI aggregation; and this included a significant increase in TSLP production.

Methods

Mice

Socs7 deficient mice were generated as described [9]. For experiments, 129/Bl6 mixed KO mice as well as wild-type 129/Bl6 F2 hybrid mice were used. All procedures were performed in accordance with the regulations of the University of Iowa Animal Care and Use Committee.

Cell culture

Mouse bone marrow derived mast cells (BMMCs) were cultured from marrow cells of the femur and tibia of 129/Bl6 *Socs7*$^{+/+}$ or *Socs7*$^{-/-}$ mice. These cells were maintained in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, 1.0 mM sodium pyruvate, nonessential amino acids (BioSource International, Camarillo, CA), 0.0035% 2-ME and 300 ng/ml recombinant mouse IL-3 (PeproTech, Rocky Hill, NJ) as described [11]. BMMCs (>95% purity as determined by toluidine blue staining) were used at 4–6 weeks of culture.

In vitro mast cell FcεRI aggregation and cytokine release

Mast cell degranulation following FcεRI aggregation was performed as described [12]. Briefly, BMMCs were seeded at 5×10^4 cells/well in 96 well flat bottom plates. For degranulation experiments, BMMC were sensitized with 100 ng/ml mouse IgE anti-DNP (Sigma-Aldrich, St. Louis, MO) for 24 h followed by 3 washes to remove excess IgE. Thirty minutes (at 37 °C) after addition of 100 ng/ml DNP-HSA (Sigma-Aldrich), *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside (Sigma-Aldrich) was added to cell supernatants and lysates (generated by the addition of 0.1% Triton X-100) for 90 min as a chromogenic substrate for *N*-acetyl-β-D-hexosaminidase. The reaction was stopped with 0.2 M glycine. Optical density was measured at 405 nm using a GENios ELISA plate reader (ReTirSoft, Inc., Toronto, Ontario, Canada). Cytokines were measured in cell culture supernatants of BMMCs seeded at 2×10^5 cells/well and sensitized for 24 h with 100 ng/ml IgE anti-DNP (Sigma-Aldrich) [12]. Following sensitization, 100 ng/ml DNP-HSA (Sigma-Aldrich) was added to BMMCs for a total of 8 h prior to supernatant collection for cytokine analysis. Mouse TNF-α, IL-6, IL-13 and TSLP were measured using DuoSet ELISA Development Systems (R&D Systems, Minneapolis, MN). Optical density was measured at 450 nm using a GENios ELISA plate reader (ReTirSoft, Inc.).

Histology of skin from *Socs7*$^{-/-}$ mice

Skin samples were obtained from 129/Bl6 *Socs7*$^{+/+}$, as well as skin disease-free and diseased 129/Bl6 *Socs7*$^{-/-}$ mice, placed in Carnoy's fixative, embedded in paraffin and mounted on glass slides and stained using toluidine blue (American Histolabs, Gaithersburg, MD). Degranulating mast cells were identified by the presence of >2 toluidine blue stained granules present within the skin tissue immediately surrounding mast cells [13]. The data presented represents the average number of mast cells ± SEM from 6 random areas per skin section from each of the 3 mice per group.

Determination of serum Ig levels

For measuring serum Ig levels, serum from 129/Bl6 *Socs7*$^{+/+}$ and disease-free 129/Bl6 *Socs7*$^{-/-}$ mice were assayed using an enzyme linked immunoabsorbent assay (ELISA). Plates were coated overnight with capture antibody for each immunoglobulin (2 μg/ml) (Pharmingen, Franklin Lakes, NJ). After blocking with 1% BSA, standards and culture supernatants were serially diluted and added to the plate. After overnight incubation, the plates were washed and biotinylated detection antibody (2 μg/ml) (Pharmingen) was added to the plate followed by incubation for 1 h at room temperature. Streptavidin alkaline phosphatase (1:1000) (Pharmingen) was bound to the biotinylated detection antibody and incubated for an additional 1 h. The signal was developed using pNPP Alkaline Phosphatase Substrate (Sigma-Aldrich) and read at 405 nm using a VersaMax Microplate reader (Molecular Devices).

Quantitative real-time PCR

Total RNA from BMMCs was isolated using Qiagen Rneasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. RNA was reverse-transcribed using the SuperScript III First-strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA). Quantitative real-time PCR was performed using