405 STAT3 activation causes IL-33 expression in normal human epidermal keratinocyte

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Introduction

IL-33, a member of IL-1 superfamily, behaves as a Th2 cytokine and is involved in the pathogenesis of inflammatory diseases, such as asthma, atopic dermatitis, and possibly, psoriasis. Serum IL-33 levels in psoriasis patients were elevated compared to that in healthy donors. Usually IL-33 is expressed in the nucleus of endothelial and epidermal cells, which can bind to chromatin in the nucleus, and it was released from necrotic cells.

We previously reported that the expression of IL-33 was induced by UVB, IL-17A and IFN γ in normal human epidermal keratinocytes (NHEKs). IFN γ activated IL-33 promoter directly through STAT1 signaling pathway, while TNF α did not activate IL-33 promoter using reporter gene assay. We also revealed that IL-33 was expressed in the nucleus of epidermal keratinocytes in the lesional skin of psoriasis. However, the detailed mechanism of IL-33 expression is still unclear.

Background

IL-33 is detected in the nucleus of epidermal keratinocytes in skin biopsy from patients with psoriasis. IL-33 is expressed mainly in the suprabasal layers of epidermis in lesional skin of psoriasis (left panel). Many cytokines are detected in lesional skin of psoriasis including IFN γ , TNF α , IL-1 and Oncostatin M (OSM). In addition, STAT3 is constitutively active in keratinocytes in lesional epidermis of psoriasis skin, and OSM is one of upstream activators of STAT3. OSM is produced by activated T-lymphocytes, monocytes and macrophages.

Figure; Immunostaining of IL-33 for lesional skin of psoriasis.

Materials and Methods

Cells: Primary normal human epidermal keratinocytes (NHEKs) were purchased from Cell applications Inc..

Medium: NHEKs were maintained in Keratinocyte SFM (GIBCO) supplemented by 40 ng/mL of bovine pituitary extract (BPE, Kyokuto) and 5 ng/mL of epidermal growth factor (EGF, R&D systems).

Oncostatin M (OSM) and Tumor necrosis factor α (TNF α): These cytokines were purchased from Wako chemical and R&D systems. Primary antibodies: anti-IL-33 (goat polyclonal, R&D systems), anti-p-STAT1, p-STAT3, STAT1, STAT3, p-p44/42, p-SAPK/JNK and SAPK/JNK were purchased from CST Japan, and anti-p44/42 was purchased from Santa Cruz Biotechnology. HRP conjugated anti-mouse and anti-rabbit IgG were purchased from CST Japan, and anti-goat IgG was from abcam.

Quantitative Polymerase Chain Reaction (qPCR): To evaluate RNA expression levels, we utilized TaqMan Gene Expression assay (Applied Biosystems).

Immuno Blotting (IB): Proteins were blotted to PVDF membranes and reacted with each primary antibody and 2nd antibody. IB images were visualized with Lumino image analyzer, ImageQuant LAS4000 (GE).

Results

Both RNA and protein of IL-33 was significantly induced by OSM dose and time dependent manner. In this study we used 50 ng/mL of OSM for stimulation of NHEKs. Induced IL-33 was presence in nucleus of NHEKs.

In order to identify key signaling cascade of OSM for induction of IL-33 in NHEKs we used inhibitors for several signal molecules. Both mRNA and protein of IL-33 was suppressed with JAK and JAK3 inhibitors, p44/42 inhibitor and EGFR inhibitors. We transfected adenovirus carrying wild type or dominant negative STAT1,3 or JAK suppresser, SOCS1,3 into NHEKs. STAT3 dominant negative and SOCS3 inhibited IL-33 expression induction by OSM after 24hr stimulation. These results suggested that IL-33 expression is controlled mainly through JAK3—STAT3 pathway, while STAT1 is not involved in the induction of IL-33 by OSM stimulation.

Usually TNF α is abundantly expressed in psoriasis patients, and anti-TNF α inhibitors are effective for psoriasis treatment. We examined whether TNF α interacts or not to IL-33 expression induced by OSM. TNF α induced expression of IL-33 mRNA at 2 to 4 hr after stimulation, while it suppressed IL-33 expression at later time points of stimulation. Combination of TNF α and OSM followed similar time course to TNF α alone, and the IL-33 expression was strongly suppressed 24 to 48 hours after stimulation.

TNF α induced IL-33 expression at early time point after stimulation, and quickly suppressed it. Our results indicated that OSM and TNF α signals did not interfere each other. In addition, OSM and TNF α induced STAT3 phosphorylation and JAK3 expression which is important to induction of IL-33. Combination of OSM and TNF α more strongly induced JAK3 and SOCS3 expression compared to each cytokine alone. These results suggested that TNF α did not directly suppress JAK3-STAT3 signaling induced by OSM directly.

Conclusion

OSM induced IL-33 expression both at mRNA and protein levels in NHEKs. These events were dependent on time after OSM stimulation and OSM concentration. IL-33 induced by OSM was expressed in nucleus of NHEKs. IL-33 induced by OSM was suppressed by JAK, p44/42 and EGFR inhibitors, and STAT3 dominant negative and SOCS3, endogenous inhibitor of JAK3. TNF α induced IL-33 at 4hr after stimulation, however at late time point of stimulation, suppressed IL-33 expression both at mRNA and protein levels. Stimulation with combination of OSM and TNF α followed similar time-course to stimulation with TNF α alone, and suppressive effect of OSM with TNF α was stronger than TNF α alone. OSM mainly induced STAT3 phosphorylation and weakly phosphorylated STAT1, while TNF α did not interfere with STAT signaling induced by OSM. TNF α did not suppress STAT3 or JAK3 expression at mRNA levels and did not induce SOCS3 expression. STAT3 signal has an important role for induction of IL-33 in NHEKs and suppression by TNF α may involve other molecules suppress IL-33 expression, such as miRNA and/or transcription suppresser molecules.