·Basic Research·

Regulation of interleukin 33/ST2 signaling of human corneal epithelium in allergic diseases

Jing Lin, Gui-Qiu Zhao, Qian Wang, Qiang Xu, Cheng-Ye Che, Li-Ting Hu, Nan Jiang, Qing Wang, Li-Li Zhang

Foundation item: National Natural Science Foundation of China (No. 81170825)

Department of Ophthalmology, the Affiliated Hospital of Medical College, Qingdao University, Qingdao 266003, Shandong Province, China

Correspondence to: Gui-Qiu Zhao. Department of Ophthalmology, the Affiliated Hospital of Medical College, Qingdao University, Qingdao 266003, Shandong Province, China. yankezhaoguiqiu@126.com

Received: 2012-11-19 Accepted: 2012-12-18

Abstract

• AIM: To identify the function of ST2 and explore the role of IL-33/ST2 signaling in regulating the pro-allergic cytokine production in human corneal epithelial cells (HCECs).

• METHODS: Human corneal tissues and cultured primary HCECs were treated with IL-33 in different concentrations without or with different inhibitors to evaluate the expression, location and signaling pathways of ST2 in regulating production of pro-allergic cytokine and chemokine. The expression of mRNA was determined by reverse transcription and real time PCR, and protein production was measured by enzyme-linked immunosorbent assay (ELISA), immunohistochemical and immunofluorescent staining. ST2 protein was detected in donor corneal epithelium, and ST2 signal was enhanced by exposure to IL-33.

• RESULTS: IL-33 significantly stimulated production of pro-allergic cytokines thymic stromal lymphopoietin (TSLP) and chemokine (CCL2, CCL20, CCL22) in HCECs at both mRNA and protein levels. These stimulated productions of pro-allergic mediators by IL-33 were blocked by ST2 antibody or soluble ST2 protein(P<0.05). Interestingly, the IκB-α inhibitor BAY11-7082 or NF-κB activation inhibitor quinazoline blocked NF-κB p65 protein nuclear translocation, and also suppressed the productions of these pro-allergic cytokines and chemokine induced by IL-33.

• CONCLUSION: These findings demonstrate that IL-33/ST2 signaling plays an important role in regulating IL-33 induced pro-allergic responses. IL-33 and ST2 could become novel molecular targets for the intervention of allergic diseases in ocular surface.

• **KEYWORDS:** ST2; interleukin 33; human; cornea; epithelium; allergic diseases; NF-κB

DOI:10.3980/j.issn.2222-3959.2013.01.05

Lin J, Zhao GQ, Wang Q, Xu Q, Che CY, Hu LT, Jiang N, Wang Q, Zhang LL. Regulation of interleukin 33/ST2 signaling of human corneal epithelium in allergic diseases. *Int J Ophthalmol* 2013;6(1):23-29

INTRODUCTION

Allergic diseases, such as seasonal allergy, asthma, and atopicdermatitis, affect up to 20% -30% of the population in industrialized countries, and up to 50% of these subjects report ocular allergic manifestations [1]. The incidence of allergies has increased steadily over the past 30 years. TH2-dominant hypersensitivity is a major contributor to allergic inflammatory disease, but the underlying mechanism of this adaptive immune disorder by corneal epithelia remains a relative mystery.

Interleukin (IL)-33 is the newest member of the IL-1 Family of cytokines and has been best characterized as a potent inducer of T helper 2 (Th2) immune responses [2]. Similar to IL-1α and high-mobility group protein B1 (HMGB-1), IL-33 appears as a cytokine with dual function, acting both as a traditional cytokine and as an intracellular nuclear factor with transcriptional regulatory properties [3]. IL-33 is expressed in various types of cells, including epithelial cells, endothelial cells, fibroblasts and smooth muscle cells [3]. IL-33 induces and activates mast cells to secrete the CC-chemokine ligand 2 and 3(CCL2 and CCL3) [4]. Epithelial- derived interleukin (IL)-33 is critical regulators of innate and adaptive immune responses associated with Th2 cytokinemediated inflammation [5]. Increasing evidence indicates that IL-33 also represents an important mediator of mucosal epithelial restoration and repair [2]. However, the pro-allergic response in corneal epithelium induced by IL-33 remains to be elucidated.

ST2 was first identified as a soluble protein in growth-stimulated fibroblasts [6]. Schmitz *et al* [7] first identified the orphan receptor "ST2" as a receptor for IL-33. After this initial report, more investigators reported the expression and function of IL-33 and ST2 in various types of cells. The ST2 gene is now known to encode at least 3 isoforms of ST2 proteins by alternative splicing: a trans-membrane receptor

ST2L; a secreted soluble ST2 (sST2) form which can serve as a decoy receptor for IL-33; and ST2V, a variant form present mainly in the gut of humans [8]. After identification of IL-33 as a novel ligand of ST2, more investigators reported the expression and function of IL-33/ST2 signaling in various types of cells. ST2/IL-33 overstimulation has been implicated in autoimmune diseases such as arthritis [9], allergic inflammation [10], and airway hyperactivity [11], demonstrating an important role of ST2 in the development of pro-allergic inflammatory pathologies [12]. This leads to activation of transcription factors such as NF-κB and AP-1 *via* TRAF6, IRAK-1/4 and MAP kinases and the production of pro-allergic mediators. However, the functions of IL-33/ST2 in epithelium, especially corneal epithelium, are not clear, although a few studies showed ST2 significantly increased inflammatory cytokines in retinal pigment epithelium (RPE) cells very recently [13]. In present study, we demonstrated, for the first time, that IL-33 stimulated the expression and production of pro-allergic cytokine and chemokine *via* ST2 mediated NF-kB signaling pathways in human corneal epithelial cells.

MATERIALS AND METHODS

Materials Cell culture plates, centrifuge tubes and other plastic ware were purchased from Becton Dickinson (Lincoln Park, NJ). Dulbecco modified Eagle medium (DMEM), Ham F-12, amphotericin B, gentamicin and 0.25% trypsin/0.03% EDTA solution were from Invitrogen-GIBCO BRL (Grand Island, NY). Fetal bovine serum (FBS) was from Hyclone (Logan, UT). Primary antibodies (mAb) against ST2 came from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescein Alexa Fluor 488 conjugated second antibodies (goat anti-mouse or anti-rabbit IgG) were from Molecular Probes (Eugene, OR). Soluble recombinant human ST2 were from Abbiotec (San Diego, CA). TSLP Rabbit antibody was from ProSci Incorporated (Poway, CA). Hydrocortisone, human EGF, cholera toxin A subunit, dimethyl sulfoxide (DMSO), Hoechst 33342 and other reagents came from Sigma (St Louis, MO). Affinity purified rabbit polyclonal antibodies (Ab) against p65 were from Santa Cruz Biotechnology (Santa Cruz, CA). Enzyme-linked immunosorbent assay (ELISA) DuoSet kits for human TSLP, CCL2, CCL20 and CCL22 were from R&D Systems (Minneapolis, MN). RNeasy Mini RNA extraction kit from Qiagen (Valencia, CA); enhanced chemiluminescence (ECL) reagents and Ready-To-Go-Primer First-Strand Beads were obtained from GE Healthcare (Piscataway, NJ); TaqMan gene expression assays and real-time PCR master mix were from Applied Biosystems (Foster City, CA).

Methods

Human corneal epithelial tissue *ex vivo* model for pro-allergic cytokine and chemokines induction A fresh corneoscleral tissue was cut into four equal-sized pieces. Each quarter of the corneoscleral tissue was placed into a well of an eight-chamber slidewith epithelial side up in 150mL of serum-free SHEM medium [14], without or with IL-33 (10ng/mL) for 24 hours in a 37℃ incubator. The corneal epithelial tissues were prepared for frozen sections for ST2 and TSLP immunohistochemical staining.

Primary human corneal epithelial culture model for pro-allergic cytokine and chemokines induction Fresh human corneoscleral tissues from donors were obtained from the Affiliated Hospital of Medical College, Qingdao University. HCECs were cultured in 12-well plates with explants of corneal limbal rims in a supplemented hormonal epidermal medium (SHEM) containing 5% FBS according to previously reported method [15]. The growth of corneal epithelial cells was carefully monitored, and only the epithelial cultures without visible fibroblast contamination were used for present study. Confluent corneal epithelial cultures were switched to serum-free SHEM and treated with IL-33 in different concentrations. Each experiment was repeated at least three times. The cells treated for 1 hour-24 hours were lysed for total RNA extraction and evaluating mRNA expression. The supernatants of the conditioned medium and the cell lysate in the cultures treated for 24-48 hours were collected and stored at -80℃ for immunoassay.

IL-33/ST2/NF-κB signaling pathway evaluation HCECs were preincubated with specific ST2 antibodies (5μg/mL), soluble recombinant human ST2 (10ng/mL) or pathway inhibitors, BAY11-7082 (10μmol/L) or NF-κB activation inhibitor (quinazoline 10μmol/L) for 1 hour before IL-33 was added for 4, 6, 24, 48 hours, respectively [16]. The cells in eight-chamber slides were fixed for NF-κB p65 immuno-fluorescent staining. The cells in 12-well plates were subjected to total RNA extraction for measuring pro-allergic cytokine TSLP and chemokine (CCL2, CCL20, CCL22) expression by RT and real-time PCR. The cultured cells treated for 24-48 hours were lysed in RIPA buffer for ELISA.

Total RNA extraction, reverse transcription (RT) and quantitative real-time PCR Total RNA was isolated from cells using a QiagenRNeasy® Mini kit according to the manufacturer's protocol, and quantified by a NanoDrop® ND-1000 Spectrophotometer and stored at -80℃. The first strand cDNA was synthesized by RT from 1μg of total RNA using Ready-To-Go You-Prime First-Strand Beads as previously described [17]. The real-time PCR was performed in aMx3005PTM system (Stratagene) with 20μL reaction volume containing 5μL of cDNA, 1μL of TaqMan® Gene Expression Assay for TSLP, CCL2, CCL20, CCL22 and 10μL Master Mix. The thermocycler parameters were 50℃ for 2 minutes, 95℃ for 10 minutes, followed by 40 cycles of 95℃ for 15 seconds and 60℃ for 1 minute. A non-template control was included to evaluate DNA contamination. The

results were analyzed by the comparative threshold cycle (CT) method and normalized by GAPDH [18].

Enzyme-linked immunosorbent assay Double-sandwich ELISA for human TSLP, CCL2, CCL20, CCL22 was performed, according to the manufacturer's protocol, to determine the concentration of TSLP, CCL2, CCL20, CCL22 protein in conditioned media and culture cell lysates from different treatments. Absorbance was read at 450nm with a reference wavelength of 570nm by a VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA).

Immunohistochemical and immunofluorescent staining Indirect immunostaining was performed according to our previously reported methods [15]. In brief, the human corneal frozen sections or corneal epithelial cells on eight chamber slides were fixed in acetone at -30℃ for 5 minutes. Cell cultures were permeabilized with 0.2% Triton X-100 in PBS at room temperature for 10 minutes. Primary goat antibodies against human ST2 (1:100, 2μg/mL), TSLP (1:1000, 1μg/mL) or rabbit antibody against human p65 (1:100, 2μg/mL) were applied for 1 hour. A donkey anti-Goat biotinylated secondary antibody (R&D Systems) and an ABC peroxidase system (Vectastain; Vector Laboratories, Burlingame, CA) were then used for histochemical staining. For fluorescent staining, AlexaFluor 488-conjugated secondary antibody was applied for 1 hour followed by propidium iodide (PI, 2μg/mL) for 5 minutes for nuclear counterstaining. Secondary antibody alone or isotypeIgG were used as the negative controls. The results were photographed with an epifluorescence microscope (Eclipse 400; Nikon, Garden City, NY) using a digital camera (DMX 1200; Nikon).

Statistical Analysis Student's *t*-test was used to compare differences between two groups. One-way ANOVA test was used to make comparisons among three or more groups, and the Dunnett's post hoc test was used to identify between group differences. *P* values <0.05 were considered statistically significant.

RESULTS

IL-33 stimulated expression and production of pro-allergic mediators by HCECs To explore the role of IL-33 in immune response by corneal epithelium, we evaluated the expression of mRNA and protein production of pro-allergic cytokine TSLP and pro-allergic chemokines (CCL2, CCL20, CCL22) in primary HCECs by RT-qPCR and ELISA, respectively. With the untreated primary HCECs as controls, the mRNA expression of TSLP, CCL2, CCL20, CCL22 was significantly induced (up to 2-3fold) in cells exposed to IL-33 (10ng/mL) for 4 hours (Figure 1A). The stimulatory effects on these pro-allergic cytokines and chemokine were shown in a dose-dependent manner in 2-50ng/mL of IL-33 (Figure 1B). These stimulatory responses were confirmed at the protein levels with 2- to 4-fold increases of TSLP, CCL2, CCL20, and CCL22 protein concentrations, respectively, secreted in the media from HCECs treated with IL-33 in 2-50ng/mL (Figure 1C).

Induction of TSLP in an *ex vivo* model of human corneal tissues To further investigate the stimulation of TSLP protein *ex vivo*, fresh human donor corneal tissues were incubated *ex vivo* with IL-33 10ng/mL for 48 hours. Immunohistochemical staining showed that TSLP protein was normally produced by corneal epithelial cells, and mainly located in the cytoplasm in the untreated corneal epithelial tissues. Stronger staining throughout multiple layers of corneal epithelium was observed in the tissues exposed to IL-33 10ng/mL for 48 hours (Figure 2).

IL-33 stimulated pro-allergic mediators *via* ST2 signaling in HCECs To investigate the cellular location and stimulation of ST2 protein *ex vivo*, fresh donor corneal tissues were incubated with IL-33 (10ng/mL) for 48 hours, followed by immunohistochemical staining. As shown in Figure 2, the ST2 protein mainly located in the cell membrane and cytoplasm in the superficial epithelial layers of normal donor corneas. Stronger immunoreactivity throughout multiple layers of corneal epithelium was observed in the tissues exposed to IL-33 for 48 hours.

The role of ST2 in corneal epithelium is largely unknown. Here we investigated whether ST2 signaling was essential in IL-33-stimulated production of pro-allergic inflammatory mediators by primary HCECs. When treated with 10ng/mL of IL-33 for 4-48 hours, the production of TSLP, CCL2, CCL20 and CCL22 was significantly increased at both mRNA (Figure 3A) and protein (Figure 3B) levels. Pre-treatment with 5μg/mL ST2 antibody or 10ng/mL of soluble recombinant human ST2 protein one hour prior IL-33, significantly suppressed the expression of TSLP, CCL2, CCL20 and CCL22 induced by 10ng/mL of IL-33 at both mRNA (Figure 3A, all $P<0.05$, $n=4$) and protein (Figure 3B, $P<0.05$ $n=4$) levels. These finding suggests that ST2 play an important role in IL-33 induces pro-allergic inflammation in HCECs.

IL-33 mediated pro-allergic responses *via* NF-κB signaling pathway in cultured HCECs *in vitro* We further investigated whether NF-κB signaling pathway is involved in IL-33/ST2 stimulated inflammatory response in HCECs. As shown in Figure 3A and B, pre-treatment of NF-κB activation inhibitor quinazoline (NF-κB-I, 10μmol/L) or IkappaB-α inhibitor BAY11-7082 (10μmol/L) significantly suppressed the mRNA expression and protein production of TSLP, CCL2, CCL20 and CCL22, stimulated by 10ng/mL of IL-33 in HCECs (Figure 3A and B). The NF-κB activation was further confirmed by immunofluorescent staining showing the translocation of NF-κB p65 protein from cytoplasm to nucleus in HCECs exposed to 10ng/mL of IL-33 (Figure 4). Interestingly, the IL-33 stimulated p65 nuclear translocation was markedly blocked by ST2 antibody

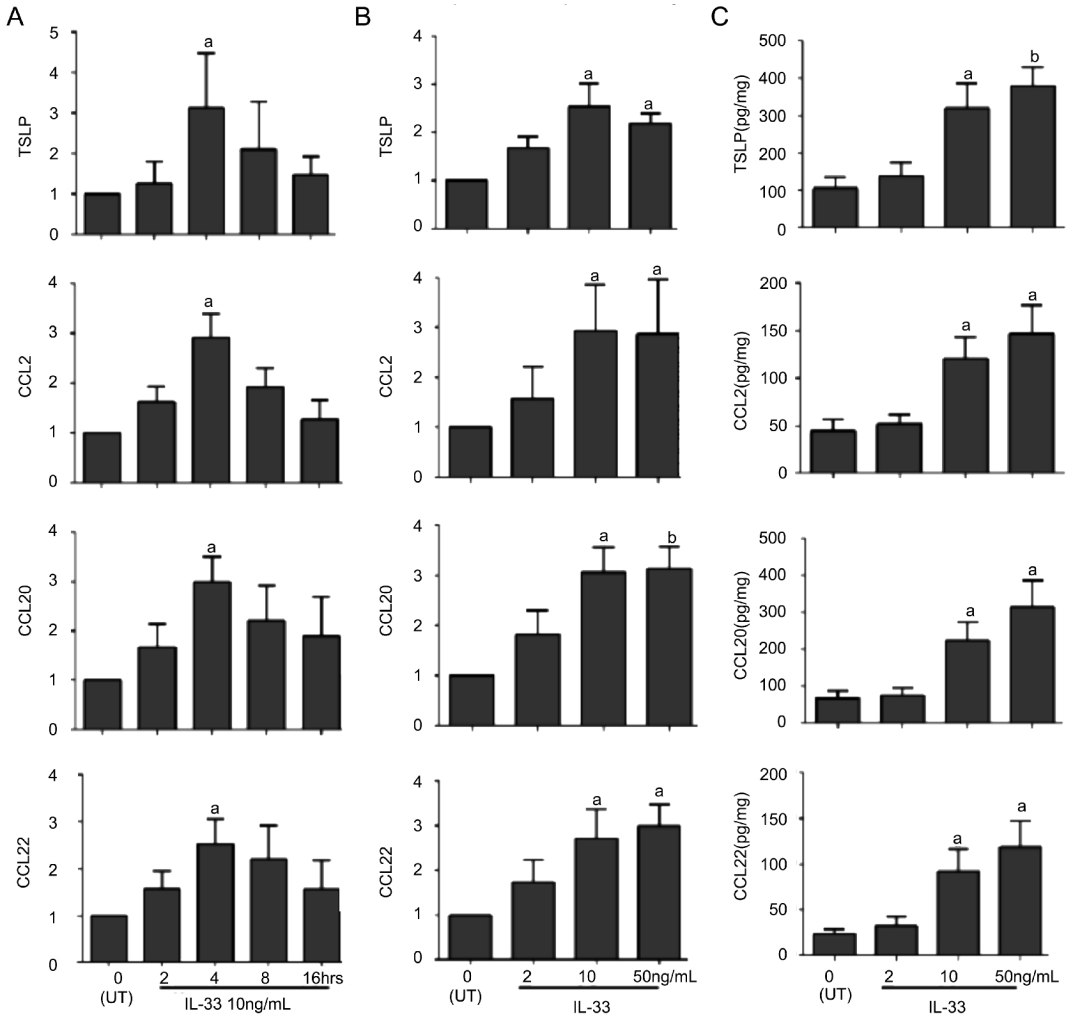

Figure 1 IL-33 induced pro-allergic mediators in HCECs with time course and dose response A: Relative fold of mRNA(Time); B: Relative fold of mRNA (Dose dependent); C: Protein in medium. The expression of pro-allergic chemokines (CCL2, CCL20, CCL22) were measured by RT-qPCR for mRNA (A, B) and by ELISA for protein levels in culture supernatants (C). Results shown are mean±SD of four independent experiments. [a] $P<0.05$; [b] $P<0.01$, $n=4$.

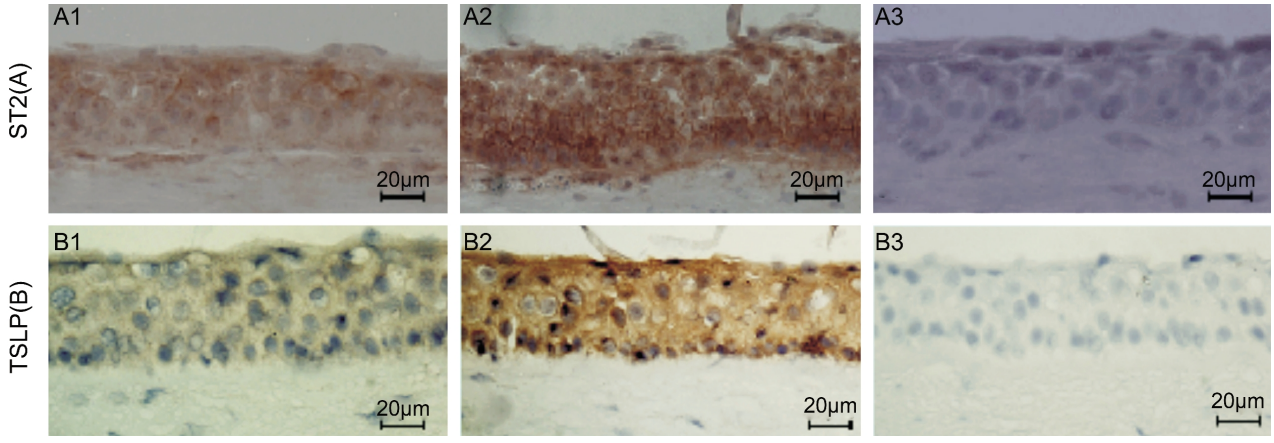

Figure 2 ST2 and TSLP were expressed by human corneal epithelium A: Representative images showing ST2 localization in *ex vivo* donor corneal tissues without (A1) or with exposure to IL-33 (10ng/mL)(A2) by immunohistochemical staining with isotypeIgG (A3) as a negative control; B: Immunohistochemical images showing TSLP protein in *ex vivo* donor corneal tissues without (B1) or with exposure to IL-33 (10ng/mL) (B2) by immunohistochemical staining with isotypeIgG (B3) as a negative control. Magnification 400×.

(ST2Ab, 5μg/mL), but not by its isotypeIgG. Furthermore, the p65 activation was also blocked by NF-κB-I (Figure 4). These results suggest that IL-33 induces inflammatory responses by HCECs *via* ST2 signaling and NF-κB activation.

The NF-κB signaling pathway also appeared to mediate the production of IL-33-inducing pro-allergic cytokines and chemokinein cultured HCECs. As shown in Figure 3A and B, NF-κB activation inhibitor quinazoline (NF-κB-I, 10μmol/L)

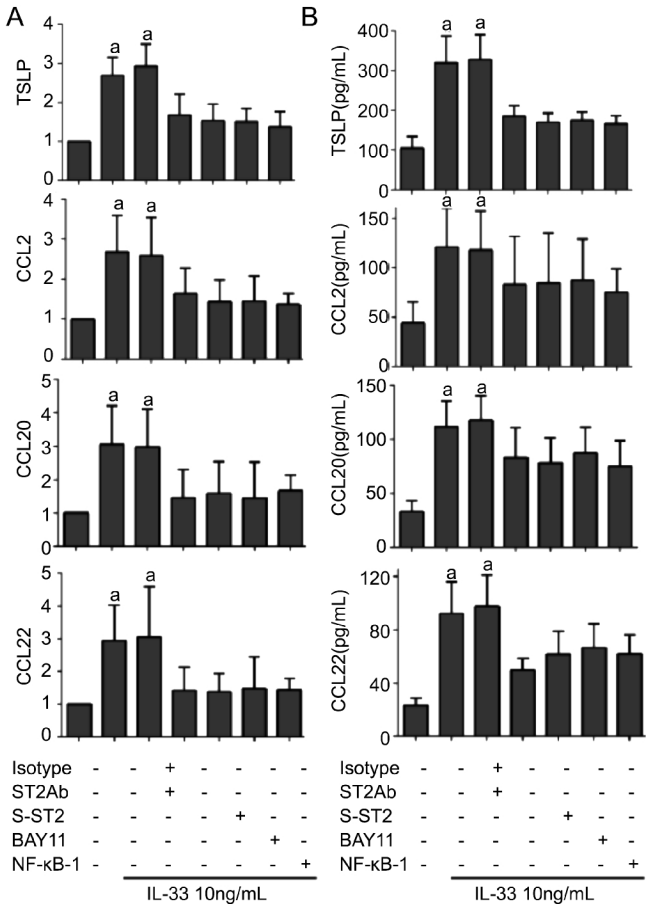

Figure 3 ST2 and NF-κB signaling pathways were involved in IL-33 induced pro-allergic response A: Relative fold of mRNA; B: Protein in medium. The HCECs were exposed to IL-33 (10ng/mL) with prior incubation in the absence or presence of isotypeIgG (5μg/mL), ST2Ab (5μg/mL), soluble ST2 protein (10ng/mL), BAY11-7082 (10μmol/L) or NF-kB activation inhibitor quinazoline (NF-kB-I, 10μmol/L) for 1 hour. The cultures treated by IL-33 for 4 hours were subjected to RT-qPCR to measure mRNA (A), the cultures treated for 48 hours were used to evaluate protein in medium supernatants by ELISA (B). Results shown are the mean±SD of four independent experiments. $^aP<0.05$; $n=4$.

or IkappaB-α inhibitor BAY11-7082 (10μmol/L) significantly suppressed the mRNA expression and protein production of pro-allergic cytokine TSLP and chemokines (CCL2, CCL20, CCL22), stimulated by 10ng/mL of IL-33 in HCECs (Figure 3A and B).The NF-κB activation by p65 was further confirmed by directly observing translocation of p65 protein from cytoplasm to nuclei of cells exposed to IL-33 10ng/mL, by immunofluorescent staining as shown in Figure 4. The stimulated p65 activation was markedly blocked byNF-κB-I (Figure 4). More interestingly, immunofluorescent staining further confirmed that the IL-33 stimulated p65 nuclear translocation was markedly blocked by ST2 antibody (ST2Ab, 5μg/mL), but not by its isotypeIgG (Figure 4). These results suggest that IL-33 induces pro-allergic responses by HCECs *via* ST2 signaling and NF-κB activation.

DISCUSSION

IL-33, a novel member of the IL-1 cytokine family, has been identified as a natural ligand of the IL-1 receptor family member ST2, which is considered to play a crucial role in allergic diseases. By binding to ST2 receptor, IL-33 can activate Th2 cells and mast cells to secrete Th2 cell-associated cytokines and chemokines that lead to severe pathological changes in mucosal organs [7]. Recent studies showed that the IL-33 and its receptor ST2 play important roles in allergic rhinitis [19]. However, the roles of IL-33 and ST2 in pro-allergic response by corneal epithelia have not been well investigated, though Matsuda *et al*[20] reported that IL-33-ST2 signaling cascade plays some roles in the pathophysiology of chronic allergic conjunctivitis through the activation of mast cells. Using fresh donor corneal tissues and primary human corneal epithelial cells, the present study revealed that ST2 was expressed on corneal epithelium and IL-33/ST2 can activate local corneal epithelial cells to secrete pro-allergic cytokines and chemokines through NF-κB signaling pathways.

It is well known that IL-33 is a novel pro-allergic cytokine which is involved in allergic inflammation [21]. It is a potent inducer of pro-allergic cytokine and chemokine produced by target cells, including mast cells [4], basophils and eosinophils [22], endothelial cells [21], Th2 lymphocytes [23], and invariant NKT and NK cells [24]. However, the pro-allergic function of IL-33 in local epithelium has not been reported especially in corneal epithelial cells. As shown in Figure 1A and C, IL-33 significantly stimulated production of pro-allergic cytokine TSLP and chemokines (CCL2, CCL20, CCL22) by HCECs at both mRNA and protein levels. In addition, the peak mRNA levels of TSLP, CCL2, CCL20, CCL22 noted at 4 hours in HCEC (Figure 1B). TSLP protein was detected to be located in the cytoplasm in the untreated corneal epithelial tissues, and it was stimulated to stronger staining throughout multiple layers of the epithelium by exposed to IL-33 in *ex vivo* donor corneal epithelium (Figure 2). The pattern of concentration-dependent IL-33 induced TSLP, CCL2, CCL20, CCL22 indicates that IL-33 is able to rapidly initiate a pro-allergic response in local corneal epithelium and plays an important role in corneal allergic disease.

ST2 molecule is a member of the IL-1 receptor family [25]. IL-33/ST2 axis can promote both Th1 and Th2 immune responses depending on the type of activated cell and microenvironment and cytokine network in damaged tissue [26]. Previous reports showed that ST2/IL-33 system activation promotes pro-allergic response that was extensively studied during the last few years. The IL-33/ST2 axis appears to play an important role in sever chronic allergic disorders, including asthma, rheumatoid, arthritis, and anaphylactic shock. IL-33/ST2 axis as the first line of defense in infection of bacterial and IL-33/ST2 signaling affects immune

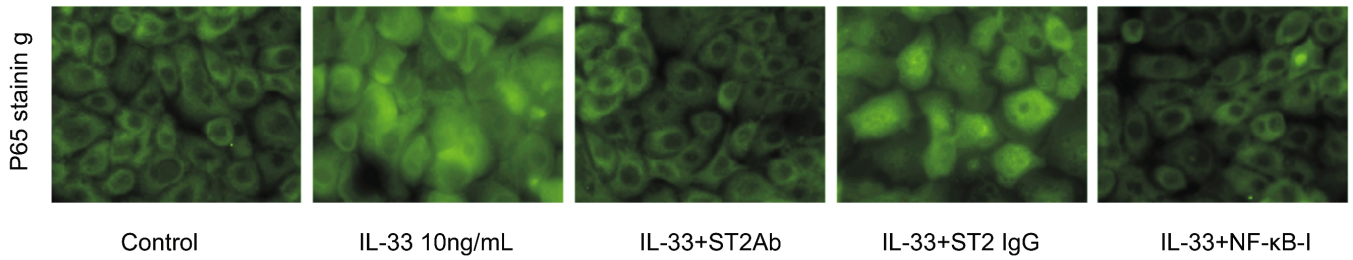

Figure 4 NF-κB activation was induced by IL-33 in HCECs The HCECs were exposed to IL-33 (10ng/mL) with prior incubation in the absence or presence of ST2Ab (5μg/mL), isotypeIgG (5μg/mL) or NF-κB activation inhibitor quinazoline (NF-κB-I, 10μmol/L) for 1 hour. The cells treated by IL-33 for 4 hours in 8-chamber slides were used for p65 immunofluorescent staining. The representative images were from three independent experiments. Magnification 400×.

response to viruses [26]. However, the function of IL-33/ST2 axis in corneal allergic disease is never been reported. In the present study, ST2 protein was detected to be located in superficial layers, and it was stimulated to multiple layers of the epithelium exposed to IL-33 in *ex vivo* donor corneal epithelium (Figure 2). Our data also showed that the expression and production of pro-allergic cytokine TSLP and chemokines (CCL2, CCL20, CCL22) that induced by IL-33 were markedly blocked by ST2 antibody and soluble-ST2 at both mRNA and protein levels in cultured corneal epithelial cells (Figure 3A and B). The findings demonstrate that ST2 is present in human corneal epithelial cells and the induction of the pro-allergic cytokine and chemokines by IL-33 is through activation of its receptor ST2 signaling. Further studies are necessary to clarify the underlying mechanism based on the importance of IL-33/ST2 in allergic disease.

NF-κB signaling pathway appears to mediate mucosal epithelial allergic inflammation [27]. NF-κB is present in the cytoplasm of resting cells as a dimer bound to an inhibitor protein (IκB) to form an inactive protein complex. To regulate gene transcription, NF-κB p65 heterodimers translocate to the nucleus from the cytoplasm [28]. NF-κB biological activity is controlled mainly by the IκB alpha and IκB beta proteins, which restrict NF-κB to the cytoplasm and inhibit its DNA binding activity. The phosphorylation of IκB, which leads to its dissociation from NF-κB protein and subsequent degradation, results in the release and translocation of NF-κB protein from cytoplasm to nucleus [29]. The present study showed that NF-κB was dramatically activated with p65 protein nuclear translocation in corneal epithelial cells exposed to IL-33 for 4 hours, demonstrated by immunofluorescent staining (Figures 4). Quinazoline, a NF-κB activation inhibitor, blocked the nuclear translocation of NF-κBp65 nuclear translocation. More interestingly, the stimulated induction of pro-allergic cytokine TSLP and chemokines (CCL2, CCL20, CCL22) by IL-33 were also markedly blocked by IkB-α inhibitor BAY11. These findings confirmed that pro-allergic cytokine TSLP and chemokines (CCL2, CCL20, CCL22) induction in HCECs by IL-33 is mediated by the NF-κB signaling pathways.

In conclusion, the present study demonstrated that ST2 is expressed in corneal epithelium and IL-33 activated pro-allergic cytokine and chemokine production in corneal epithelial cells through ST2 and NF-κB signaling pathways. The findings demonstrated that IL-33/ST2 axis plays an important role in ocular surface pro-allergic inflammatory diseases. Furthermore, IL-33 and ST2 could become novel molecular targets for the intervention of allergic diseases in ocular surface.

REFERENCES

1 Stern ME, Siemasko KF, Niederkorn JY. The Th1/Th2 paradigm in ocular allergy. *Curr Opin Allergy Clin Immunol* 2005;5(5):446-450

2 Lopetuso LR, Scaldaferri F, Pizarro TT. Emerging role of the interleukin (IL)-33/ST2 axis in gut mucosal wound healing and fibrosis. *Fibrogenesis Tissue Repair* 2012;5(1):18

3 Miller AM, Liew FY. The IL-33/ST2 pathway: a new therapeutic target in cardiovascular disease. *Pharmacol Ther* 2011;131(2):179-186

4 Ali S, Huber M, Kollewe C, Bischoff SC, Falk W, Martin MU. IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. *Proc Natl Acad Sci U S A* 2007;104 (47): 18660-18665

5 Nomura K, Kojima T, Fuchimoto J, Obata K, Keira T, Himi T, Sawada N. Regulation of interleukin-33 and thymic stromal lymphopoietin in human nasal fibroblasts by proinflammatory cytokines. *Laryngoscope* 2012;122(6): 1185-1192

6 Tominaga S. ST2 gene: a gene that is induced by growth stimulation and encoding a product highly similar to the interleukin 1 receptors. *Seikagaku* 1995;67(5):356-364

7 Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals *via* the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23(5):479-490

8 Tago K, Noda T, Hayakawa M, Iwahana H, Yanagisawa K, Yashiro T, Tominaga S. Tissue distribution and subcellular localization of a variant form of the human ST2 gene product, ST2V. *Biochem Biophys Res Commun* 2001;285(5):1377-1383

9 Palmer G, Talabot-Ayer D, Lamacchia C, Toy D, Seemayer CA, Viatte S, Finckh A, Smith DE, Gabay C. Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. *Arthritis Rheum* 2009;60 (3):738-749

10 Kearley J, Buckland KF, Mathie SA, Lloyd CM. Resolution of allergic inflammation and airway hyperre activity is dependent upon disruption of

the T1/ST2-IL-33 pathway. *Am J Respir Crit Care Med* 2009;179 (9): 772-781

11 Mangan NE, Dasvarma A, McKenzie AN, Fallon PG. T1/ST2 expression on Th2 cells negatively regulates allergic pulmonary inflammation. *Eur J Immunol* 2007;37(5):1302-1312

12 Beltran CJ, Nunez LE, Diaz-Jimenez D, Farfan N, Candia E, Heine C, Lopez F, Gonzalez MJ, Quera R, Hermoso MA. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2010;16(7):1097-1107

13 Kociok N, Heppekausen H, Schraermeyer U, Esser P, Thumann G, Grisanti S, Heimann K. The mRNA expression of cytokines and their receptors in cultured iris pigment epithelial cells: a comparison with retinal pigment epithelial cells. *Exp Eye Res* 1998;67(2):237-250

14 Ma P, Wang Z, Pflugfelder SC, Li DQ. Toll-like receptors mediate induction of peptidoglycan recognition proteins in human corneal epithelial cells. *Exp Eye Res* 2010;90(1):130-136

15 Kim HS, Jun S, X, de Paiva CS, Chen Z, Pflugfelder SC, Li DQ. Phenotypic characterization of human corneal epithelial cells expanded *ex vivo* from limbal explant and single cell cultures. *Exp Eye Res* 2004;79(1): 41-49

16 Zhang L, Lu R, Zhao G, Pflugfelder SC, Li DQ. TLR-mediated induction of pro-allergic cytokine IL-33 in ocular mucosal epithelium. *Int J Biochem Cell Biol* 2011;43(9):1383-1391

17 Luo L, Li D-Q, Doshi A, Farley W, Corrales RM, Pflugfelder SC. Experimental dry eye stimulates production of inflammatory cytokines and MMP-9 and activates MAPK signaling pathways on the ocular surface. *Invest Ophthalmol Vis Sci* 2004;45(12):4293-4301

18 de Paiva CS, Corrales RM, Villarreal AL, Farley WJ, Li D-Q, Stern ME, Pflugfelder SC. Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. *Exp Eye Res* 2006;83(3):526-535

19 Kamekura R, Kojima T, Takano K, Go M, Sawada N, Himi T. The role of IL-33 and its receptor ST2 in human nasal epithelium with allergic rhinitis. *Clin Exp Allergy* 2012;42(2):218-228

20 Matsuda A, Okayama Y, Terai N, Yokoi N, Ebihara N, Tanioka H, Kawasaki S, Inatomi T, Katoh N, Ueda E,Hamuro J, Murakami A, Kinoshita S. The role of interleukin-33 in chronic allergic conjunctivitis. *Invest Ophthalmol Vis Sci* 2009;50(10):4646-4652

21 Matsuba-Kitamura S, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Taki Y, Muto T, Ikeda T, Mimura O, Nakanishi K. Contribution of IL-33 to induction and augmentation of experimental allergic conjunctivitis. *Int Immunol* 2010;22(6):479-489

22 Hirano M, Ogita-Nakanishi H, Miyachi W, Hannya N, Yamamoto-Kimoto Y, Sakurai K, Miyoshi-Higashino M, Tashiro-Yamaji J, Kato R, Ijiri Y, Tanaka K, Kanazawa A, Terada T, Kawata R, Takenaka H, Kubota T, Yoshida R. Essential role of macrophages in the initiation of allergic rhinitis in mice sensitized intranasally once with cedar pollen: regulation of class switching of immunoglobulin in B cells by controlling interleukin-4 production in T cells of submandibular lymph nodes. *Microbiol Immunol* 2012;56:392-405

23 Kool M, Hammad H, Lambrecht BN. Cellular networks controlling Th2 polarization in allergy and immunity. *F1000 Biol Rep* 2012;4:6

24 Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int Immunol* 2008;20(8):1019-1030

25 Yanai M, Sato K, Aoki N, Takiyama Y, Oikawa K, Kobayashi H, Kimura S, Harabuchi Y, Tateno M. The role of CCL22/macrophage-derived chemokine in allergic rhinitis. *Clin Immunol* 2007;125(3):291-298

26 Matsushita H, Ohta S, Shiraishi H, Suzuki S, Arima K, Toda S, Tanaka H, Nagai H, Kimoto M, Inokuchi A, Izuhara K. Endotoxin tolerance attenuates airway allergic inflammation in model mice by suppression of the T-cell stimulatory effect of dendritic cells. *Int Immunol* 2010;22 (9): 739-747

27 Bian F, Qi H, Ma P, Zhang L, Yoon KC, Pflugfelder SC, Li DQ. An immunoprotective privilege of corneal epithelial stem cells against Th17 inflammatory stress by producing glial cell-derived neurotrophic factor. *Stem Cells* 2010;28(12):2172-2181

28 Dixit V, Mak TW. NF-kappaB signaling. Many roads lead to madrid. *Cell* 2002;111(5):615-619

29 Oshikawa K, Yanagisawa K, Tominaga S, Sugiyama Y. Expression and function of the ST2 gene in a murine model of allergic airway inflammation. *Clin Exp Allergy* 2002;32(10):1520-1526