TSLP and Downstream Molecules in Experimental Mouse Allergic Conjunctivitis

Xiaofen Zheng,^{1,2} Ping Ma,^{1,3} Cintia S. de Paiva,¹ Matthew A. Cunningham,¹ Cindy S. Hwang,¹ Stephen C. Pflugfelder,¹ and De-Quan Li¹

PURPOSE. To explore the potential role of thymic stromal lymphopoietin (TSLP) and its downstream molecules in the development of ocular allergic inflammation using a short ragweed (SRW)-induced mouse model of allergic conjunctivitis (AC).

METHODS. BALB/c mice were topically challenged with SRW pollen after they were sensitized with SRW in the footpad. After the last SRW challenge, the corneal epithelium, conjunctiva, and cervical lymph nodes were harvested for total RNA extraction and gene expression by RT and real-time PCR, and whole eye globes were collected to make cryosections for immunohistochemical staining.

RESULTS. Repeated topical challenges with SRW allergen generated typical signs of AC in mice. Compared with the untreated controls, TSLP mRNA expression and immunoreactivity were significantly increased in the corneal and conjunctival epithelia of SRW-induced AC mice. CD11c⁺ and OX40L⁺ immunoreactive cells largely infiltrated the conjunctiva with increased mRNA levels of CD11c, TSLPR, and OX40L detected in the corneal epithelium, conjunctiva, and cervical lymph nodes. CD4⁺ Th2 cell infiltration was evidenced by increased levels of mRNA and immunoreactivity of CD4, IL-4, IL-5, and IL-13 in the ocular surface, mainly in the conjunctiva, accompanied by increased expression of OX40, STAT6, and GATA3, in AC mice. The maturation of immature DCs was observed with the use of TSLP containing conditioned media from corneal epithelial cultures exposed to polyI:C, which stimulates TSLP production.

CONCLUSIONS. This study provides new findings regarding the role of local mucosal epithelial cells in the initiation of ocular allergic inflammation by producing a novel proallergic cytokine, TSLP, which activates dendritic cells to prime Th2 differentiation and allergic inflammation through the TSLP-TSLPR and OX40L-OX40 signaling pathway. (*Invest Ophthalmol Vis Sci.* 2010;51:3076–3082) DOI:10.1167/iovs.09-4122

Allergic conjunctivitis (AC) is one of the most common ocular surface diseases. Studies^{1–3} have reported that 20% to 30% of the populations in industrialized countries such as

the United States have experienced allergies, with 40% to 60% of these persons reporting ocular allergies. The incidence of allergies, including allergic conjunctivitis, has increased steadily in the past 30 years. The disease ranges in severity from mild forms, such as seasonal and perennial AC, which can still interfere significantly with quality of life, to severe cases, such as vernal keratoconjunctivitis⁴ and atopic keratoconjunctivitis,⁵ which may be complicated by corneal damage and may have the potential to cause permanent vision loss. AC is an abnormal immune-hypersensitivity response to allergens. It is characterized by IgE-mediated or T-lymphocyte-mediated immune hypersensitivity reactions that lead to an immune response. Allergen-specific T helper (Th) 2 type lymphocytes and their cytokines play important roles in the immunopathophysiology of allergic disorders because of their ability to produce IL-4 and IL-5, which are involved in IgE production and eosinophil activation, respectively (for reviews see Refs. 1, 6, 7).

Regulation of the development of Th2-type allergic inflammation locally at mucosal surfaces was a relative mystery until studies identified a novel proallergic molecule, thymic stromal lymphopoietin (TSLP).^{8–10} TSLP, an epithelium-derived cytokine, can strongly activate dendritic cells through interaction with the TSLP receptor (TSLPR) expressed by dendritic cells^{11,12} to induce an inflammatory Th2-type response and to initiate allergic inflammation.^{13,14} TSLP is produced primarily by epithelial cells in the lungs, gut, and skin, though fibroblasts, smooth muscle cells, and mast cells all have the potential to produce TSLP.^{8,15} Recent work has shown increased TSLP levels at sites of allergic inflammation. For example, airway epithelia of patients with asthma showed increased TSLP expression, supporting a role for TSLP in promoting Th2-type allergic inflammation. TSLP-treated dendritic cells express OX40 ligand (OX40L), which interacts with OX40 to prime CD4⁺ T cells to produce the proallergic cytokines IL-4, IL-13, and IL-5.^{8,13,16,17} TSLP was found to be highly expressed by keratinocytes in skin lesions of atopic dermatitis and was associated with dendritic cell activation in situ.^{18–20} These studies have demonstrated that TSLP plays an important role in the initiation and maintenance of the allergic immune response in atopic dermatitis and asthma.^{19,21} TSLP may become an important biomarker and therapeutic target for the intervention of allergic inflammatory responses.^{16,22,23}

Asthma, atopic dermatitis, and AC form the triad of common atopic IgE-dependent diseases.²⁴ Patients with one member of the triad often show symptoms of one or both of the other members, suggesting a common genetic or initiating element in these diseases. We have explored the expression and regulation of TSLP in human corneal epithelium and demonstrated that TSLP links innate and adaptive immune responses through toll-like receptors and Th2 cytokines.²⁵ Ueta et al.²⁶ have shown that TSLP is induced at mRNA and protein levels by the TLR3 ligand polyI:C in human conjunctival epithelial cells. However, the role of TSLP in ocular allergic diseases has not been reported. In the present study, we sought to

From the ¹Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas; ²Shanxi Eye Hospital, Taiyuan, Shanxi, China; and ³Provincial Hospital, affiliated with Shandong University, Jinan, China.

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Corresponding author: De-Quan Li, Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, 6565 Fannin Street, NC-205, Houston, TX 77030; dequanl@bcm.tmc.edu.

investigate the expression of TSLP and its downstream molecules in the allergic inflammation cascade using a short ragweed (SRW) pollen-induced AC murine model.

MATERIALS AND METHODS

Animals and Induction of Experimental Allergic Conjunctivitis

This animal research protocol was approved by the Center for Comparative Medicine at Baylor College of Medicine, and it conformed to the standards in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. BALB/c mice of both sexes, ranging from 6 to 8 weeks of age, were purchased from the Jackson Laboratory (Bar Harbor, ME).

The experimental mouse AC was induced using previously reported methods^{27,28} with modifications. In brief, BALB/c mice were immunized with 50 μ g SRW pollen (*Ambrosia artemisiifolia*; International Biologicals, Piedmont, OK) in 5 mg alum (Imject; Pierce Biotechnology, Rockford, IL) by footpad injection on day 0. Allergic conjunctivitis was induced by a multihit topical challenge method, in which immunized mice were given topical applications of 1.5 mg SRW pollen suspended in 10 μ L phosphate-buffered saline (PBS), pH 7.2, into each eye once a day from days 10 to 12. Animals were examined clinically for signs of immediate hypersensitivity responses 20 minutes after each topical challenge with SRW pollen. A clinical scoring scheme, similar to that described by Magone et al.,²⁷ was used to evaluate chemosis, conjunctival redness, lid edema, and tearing. Each parameter was graded on a scale ranging from 0 to 3+. On day 13, 24 hours after the last SRW challenge, the corneal epithelium, conjunctiva, whole eyes, and cervical lymph nodes were harvested for gene expression assays and histopathologic studies. Untreated mice were used as normal controls. The mice treated with alum alone without SRW were not included as control based on the results from our preliminary studies (see Supplementary Data, <http://www.iovs.org/cgi/content/full/51/6/3076/DC1>).

Total RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR

Ocular tissues were collected from normal and AC mice, as previously described.^{29,30} The corneal epithelium was scraped and directly lysed into a guanidine isothiocyanate-containing buffer (Buffer RLT; RNeasy kit; Qiagen, Valencia, CA). The conjunctiva tissue was excised, and its epithelium was dissolved in the lysis buffer (Buffer RLT; Qiagen) without homogenizer to prevent stromal cell contamination. Superficial cervical lymph nodes (CLNs) were surgically excised, smashed between two sterile frosted glass slides, and collected in the lysis buffer. Each sample was pooled with tissues from four eyes/two mice/group in each experiment, and the same experiments were repeated four times. Total RNA was extracted (RNeasy Micro or Mini kit; Qiagen) according to the manufacturer's instructions, quantified by a spectrophotometer (NanoDrop ND-1000; Thermo Scientific, Waltham, MA), and stored at -80°C before use. First-strand cDNA was synthesized by M-MuLV reverse transcription with random hexamers (Ready-To-Go You-Prime First-Strand Beads; GE Healthcare, Arlington Heights, NJ), as previously described.^{29,30}

Real-time PCR was performed with specific primers and probes (using TaqMan Gene Expression Assays and TaqMan Gene Expression Master Mix [Applied Biosystems, Foster City, CA] in a QPCR System [Mx3005P; Stratagene, La Jolla, CA]). IDs (TaqMan Gene Expression Assay IDs; Applied Biosystems) for murine GAPDH, TSLP, TSLPR, OX40L, OX40, CD4, CD11c, IL-4, IL-5, IL-13, GATA3, and STAT6 were Mm99999915, Mm00498739, Mm00497362, Mm00442039, Mm00437214, Mm00442754, Mm00498698, Mm00445259, Mm009999063, Mm00434204, Mm00484683, and Mm01160477, respectively. Results were analyzed by the comparative threshold cycle (C_t) method³¹ and normalized by GAPDH, as previously reported.^{30,32}

Immunohistochemical Staining

The eyes and lids of mice in each group were excised, embedded in optimal cutting temperature compound (VWR, Suwanee, GA), and flash-frozen in liquid nitrogen. Sagittal 10- μ m cryosections from mouse globes were cut with a cryostat (HM 500; Micron, Waldorf, Germany), placed on glass slides, and stored at -80°C before use.

Immunohistochemical staining was performed as previously described.^{33,34} In brief, tissue sections were fixed with cold acetone at -20°C for 5 minutes and were treated with 0.3% H_2O_2 for 10 minutes to quench endogenous peroxidases. The sections were sequentially blocked with avidin-biotin block (Vector Laboratories, Burlingame, CA) for 10 minutes each. After the reaction was blocked with 20% normal goat serum or donkey serum in PBS for 45 minutes, primary antibody was applied and incubated for 1 hour at room temperature. The primary antibodies used for this study included rat anti-mouse CD4 (clone H129.9, 10 μ g/mL), hamster anti-mouse CD11c (clone HL3, 10 μ g/mL), and rat anti-mouse IL-4 (clone 11B11, 20 μ g/mL) from BD PharMingen (San Jose, CA); rat anti-mouse OX40L (clone RM134L, 10 μ g/mL) from Biolegend (San Diego, CA); rabbit anti-human TSLP (1 μ g/mL) from ProSci Incorporated (Poway, CA); goat anti-mouse TSLPR (S-18, 2 μ g/mL) and OX40 (A-20, 2 μ g/mL) from Santa Cruz Biotechnology (Santa Cruz, CA); and rabbit anti-human IL-13 (1:600) from ABD Serotec (Raleigh, NC). After extensive washing, the sections were incubated with appropriate biotinylated secondary antibodies (all from BD PharMingen) and streptavidin biotin (Vectastain ABC Kit; Vector Laboratories), according to the manufacturer's protocol. The samples were incubated for 5 to 9 minutes with diaminobenzidine (DAB) or peroxidase substrate (NovaRed; Vector Laboratories) to give a brown or red stain (optimized for each antibody) and were counterstained with Mayer's hematoxylin. Secondary antibody alone and appropriate isotype IgG (BD PharMingen) were used as controls. Sections from four different eyes per group were examined and photographed with a microscope equipped with a digital camera (Eclipse E400 with a DS-Fi1; Nikon, Tokyo, Japan).

Activation of Immature Dendritic Cells by Corneal Epithelium-Derived TSLP In Vitro

To evaluate the interactions between corneal epithelial cell-derived TSLP and dendritic cells, the human leukemia monocyte line THP-1³⁵ from ATCC (Manassas, VA) were cultured in RPMI 1640 medium with 10% FBS and induced into CD11c⁺ immature dendritic cells (iDCs) with rhIL-4 (1500 IU/mL) and rhGM-CSF (1500 IU/mL) for 5 days. The induced iDCs were used for bioactivation assay with recombinant human TSLP protein (as positive control) or the supernatants of conditioned media from corneal epithelial cultures treated with polyI:C to induce TSLP, as previously reported,²⁵ with or without TSLP-neutralizing rabbit antibody or isotype-matched IgG1 mAb (negative control). All cytokines and the detection kit (TSLP ELISA kit) were from R&D Systems (Minneapolis, MN) with the exception of TSLP rabbit antibody, which was from ProSci Incorporated. Flow cytometry was used to analyze the cell surface phenotype markers CD11c, CD80, and CD86 with antibodies from BD PharMingen.

Statistical Analysis

Student's *t*-test was used to evaluate statistical significance between the two groups (AC mice vs. untreated controls). $P \leq 0.05$ was considered statistically significant.

RESULTS

Effect of Topical SRW Allergen Challenge on Ocular Allergic Inflammation in SRW-Sensitized Mice

Ten days after immunization with SRW in alum, the BALB/c mice ($n = 20$) were challenged with 1.5 mg SRW in 10 μ L PBS into each eye, with untreated normal mice ($n = 16$) as controls. Clinical observations revealed typical symptoms of aller-

FIGURE 1. TSLP expression and immunoreactivity in corneal (CN) and conjunctival (CJ) epithelia of SRW-induced AC, with untreated mice as controls. (A) TSLP mRNA expression levels are presented as relative fold in CN and CJ of AC mice over the controls, which were evaluated by RT and real-time PCR using gene expression assay, with GAPDH as an internal control. * $P < 0.05$; $n = 4$. (B) Representative images of TSLP immunohistochemical staining of corneal and conjunctival tissue of AC and untreated control mice, with isotype IgG as a negative control.

gic conjunctivitis in the treated mice; which mice developed lid edema, conjunctival redness, chemosis, and tearing; and which mice frequently scratched their eyelids. These symptoms, signs, and behavioral changes were detected within 20 minutes of topical challenge with SRW pollen and persisted at least 8 hours. Some mice developed signs that persisted until the next challenge 24 hours later (data not shown).

Upregulation of TSLP Expression by Ocular Epithelia after Topical Allergen Challenge

To determine whether TSLP production was stimulated in experimental allergic conjunctivitis, we assessed TSLP expression by ocular surface epithelia in SRW-sensitized mice after three hits of daily topical challenges. Evaluated by RT and real-time PCR, TSLP mRNA expression was found to be upregulated in the corneal and conjunctival epithelia from mice sensitized and challenged with SRW compared with untreated normal controls (Fig. 1A). Immunohistochemical staining confirmed an increase in TSLP production in the eyes with AC. As shown in Figure 1B, the corneal and conjunctival tissues of AC mice displayed stronger TSLP staining throughout the entire epithelium than did those of untreated controls. These data indicate that TSLP mRNA expression and

protein production by ocular surface epithelia increase in this experimental AC model, suggesting possible involvement of TSLP in allergic development.

Accumulation of Activated Dendritic Cells that Produce OX40L in Ocular Surface Tissue in Mice with Allergic Conjunctivitis

The accumulation of dendritic cells in ocular surface tissues in this model was detected by semiquantitative real-time PCR and immunostaining for the dendritic marker CD11c. RT and real-time PCR showed increased levels of CD11c mRNA expression in corneal and conjunctival tissues from SRW-challenged mice compared with normal controls (Fig. 2A). Immunohistochemical staining further showed CD11c-positive DCs accumulating in the allergic ocular surface tissues, primarily in the stroma subjacent to the conjunctival epithelia (Fig. 2B). We also investigated the expression of OX40L, a factor upregulated by TSLP, and TSLPR in ocular surface and cervical superficial lymph nodes. Significantly higher levels of OX40L and TSLPR mRNA transcripts were detected in corneal and conjunctival tissues from the AC group than from controls (Fig. 2A). To confirm the PCR

FIGURE 2. mRNA expression and immunoreactivity of CD11c, TSLPR, and OX40L in cornea (CN), conjunctiva (CJ), and CLN of SRW-induced AC, with untreated mice as controls. (A) mRNA expression levels of CD11c, TSLPR, and OX40L were presented as relative fold in the CN, CJ, and CLN of AC mice over controls, which were evaluated by RT and real-time PCR using gene expression assay with GAPDH as an internal control. * $P < 0.05$; ** $P < 0.01$; $n = 4$. (B) Representative images of immunohistochemical staining for CD11c and OX40L in corneal and conjunctival tissue of AC and untreated control mice, with isotype IgG as a negative control. Arrows: positive staining.

FIGURE 3. mRNA expression or immunoreactivity of CD4, IL-4, IL-5, IL-13, OX40, STAT6, and GATA3 in cornea (CN), conjunctiva (CJ), and CLN of SRW-induced AC, with untreated mice as controls. (A) mRNA expression levels of CD4, IL-4, IL-5, IL-13, OX40, STAT6, and GATA3 are presented as relative fold in the CN, CJ, and CLN of AC mice over controls, which were evaluated by RT and real-time PCR using gene expression assay, with GAPDH as an internal control. * $P < 0.05$; ** $P < 0.01$; $n = 4$. (B) Representative images of immunohistochemical staining for CD4, IL-4, IL-13, and OX40 in corneal or conjunctival tissue of AC and untreated control mice, with isotype IgG as a negative control. Arrows: positive staining.

results, OX40L protein was immunodetected in corneal and conjunctival tissues of eye sections obtained from AC and normal mice (Fig. 2B). More OX40L-immunopositive cells were observed in the conjunctival stroma, localized similarly to CD11c⁺ cells, of AC mice than of controls. Interestingly, the mRNA levels of CD11c, OX40L, and TSLPR expression in CLNs increased significantly in AC mice over the controls (Fig. 2A). These results suggest that ocular surface epithelia-derived TSLP activates ocular surface DCs that migrate to the regional lymph nodes.

Th2-Dominated Pathway on Ocular Surface in Mice with Allergic Conjunctivitis

The infiltration of T-lymphocytes in allergic ocular surface was evidenced by increased CD4 mRNA expression (Fig. 3A) and a

marked increase in the number of CD4 immunopositive cells in AC ocular tissues, especially in the conjunctiva (Fig. 3B), compared with untreated control mice. These CD4⁺ T cells appear to be from the Th2 lineage because the three key Th2 cytokines—IL-4, IL-5, and IL-13—were all found to be expressed at significantly higher levels in corneal and conjunctival tissues of the AC group than of the controls (Fig. 3A). Only IL-4 mRNA was not detected in the corneal epithelium, indicating fewer IL-4-producing Th2 cells infiltrated the cornea. Immunostaining data confirmed that the IL-4- and IL-13-producing Th2 cells primarily infiltrated the conjunctival stroma. To determine the factors responsible for Th2 differentiation, the expression of OX40, STAT6, and GATA3 was evaluated. The level of OX40 mRNA (Fig. 3A) and the number of OX40-immunore-

FIGURE 4. Representative flow cytometry histograms showing that epithelial TSLP promotes DC maturation. CD11c⁺ iDCs were induced from the THP-1 lymphocyte line. The maturation of the iDCs to CD80⁺ and CD86⁺ mature DCs was evaluated by treatment with conditioned media (CM) from corneal epithelial cultures exposed to polyI:C (50 μ g/mL), without or with TSLP-neutralizing rabbit antibody (CM+TSLP Ab) or isotype IgG1 (CM+Isotype), and an exogenous rhTSLP (10 ng/mL) was used as positive control. The number on the top-right corner of each plot indicates the percentage of CD80⁺ or CD86⁺ cells.

active cells (Fig. 3B) were significantly increased, primarily in the conjunctiva of AC mice compared with the control group. Transcriptional factor GATA3 mRNA transcripts were higher in allergic conjunctiva, but there was no difference in STAT6 expression in cornea and conjunctiva between the AC and the untreated control group. mRNA levels of all Th2 cytokines and OX40, as well as the transcription factors STAT6 and GATA3, in CLNs increased significantly in AC mice over the controls (Fig. 3A). These results suggest that DC-produced OX40L primed the Th2 differentiation from naive CD4⁺ T cells in the ocular surface tissues and cervical lymph nodes of AC mice.

Maturation of Immature Dendritic Cells Activated by Corneal Epithelial Cell-Derived TSLP In Vitro

An in vitro culture model was used to evaluate a direct effect of corneal epithelium-derived TSLP on the maturation of CD11c⁺ iDCs induced from the human leukemia monocyte line THP-1. As shown in Figure 4, exogenous TSLP and conditioned media (containing 300–500 pg/mL TSLP, as measured with an ELISA kit²⁵; data not shown) from corneal epithelial cultures treated with polyI:C in the RPMI 1640 serum-free medium could activate CD11c⁺ iDCs to be mature DCs with >50% CD80⁺ and 80% CD86⁺ cells, an effect similar to that of exogenous rhTSLP (10 ng/mL) added in the iDC culture medium as positive control. Given that the results were limited to CD80 and CD86 markers, further studies are necessary to identify other effects of epithelial TSLP on DC activation.

DISCUSSION

SRW pollen is a clinically important airborne allergen in North America and one of the major causes of allergic conjunctivitis. An animal model of allergic conjunctivitis was first established by Magone et al.²⁷ in 1998 using a single topical challenge with this allergen in SRW-sensitized mice. This murine model incorporates the clinical, cellular, and humoral parameters of aller-

gic conjunctivitis, including a ragweed-induced Th2-type cytokine production by lymphocytes. This model was modified by Stern et al.²⁸ using repeated ocular exposure to SRW, which resulted in the development of late-phase conjunctival inflammation and displayed parameters indicative of Th2 allergic conjunctivitis through chronic ocular exposure to SRW allergens. Using the same repeated ocular exposure to SRW for 3 consecutive days, we were able to induce allergic conjunctivitis in SRW-sensitized mice with symptoms and signs similar to those of the previous report.²⁸ Twenty-four hours after the third SRW challenge, the corneal epithelium, conjunctiva, whole eyes, and cervical lymph nodes were carefully collected to evaluate TSLP expression in this AC model. Interestingly, we found a significant increase in TSLP mRNA expression and protein immunoreactivity in the corneal epithelial and conjunctival tissue of AC mice compared with untreated controls (Fig. 1).

TSLP, an IL-7-like cytokine, is expressed by keratinocytes and other epithelial cells⁸ and acts through a receptor composed of a heterodimer of the IL-7 receptor alpha (IL-7R α) chain and a unique TSLP receptor chain (TSLPR) resembling the cytokine receptor common gamma chain. TSLPR is expressed on a variety of cells, including DCs,^{11,12} and TSLP drives the development of Th2 responses through its activation of CD11c⁺ DCs, which then promote Th2 differentiation of naive T cells and secrete Th2-attracting chemokines⁸ through their production of OX40L.^{36,37} We observed prominent infiltration of CD11c⁺ and OX40L⁺ immunoreactive cells in the pollen-challenged ocular surface of SRW-sensitized mice, accompanied by increased mRNA levels of CD11c, TSLPR, and OX40L in the corneal epithelia, conjunctiva, and cervical lymph nodes compared with controls (Fig. 2). These data indicate that the accumulated CD11c⁺ DCs were activated to produce OX40L, possibly through TSLPR activation by their ocular epithelia-derived ligand TSLP. Our findings suggest that

TSLP-activated DCs may play a central role in the development of allergic conjunctivitis.

The first step in the adaptive immune pathway is the process of activating the naive CD4⁺ T-lymphocytes, which require two signals to proliferate and differentiate into effector T cells and memory T-lymphocytes. The first signal is triggered by recognition of the peptide-MHC complex by TCR on CD4⁺ T-lymphocytes, and the second signal involves costimulatory interactions.³⁸ OX40 (CD134), a member of the tumor necrosis factor receptor family, is a principal costimulatory receptor that is not constitutively expressed on naive T cells but is induced after antigen recognition.³⁹ Recent studies^{40,41} demonstrate that the interaction between OX40 and OX40L signaling directly induces Th2 lineage commitment by targeting nuclear translocation of nuclear factor of activated T cells c1 (NFATc1), which then triggers IL-4 production and IL-4-dependent GATA-3 transcription. STAT6 is a critical transcriptional factor that regulates IL-4-mediated Th2 immune responses.^{42,43} In this animal model, we have shown CD4⁺ Th2 cell infiltration evidenced by increased expression and immunoreactivity of CD4, IL-4, IL-5, and IL-13 on the ocular surface, especially in the conjunctival tissues. Increased expression of OX40, STAT6, and GATA3 by the corneal epithelium, conjunctiva, and cervical lymph nodes provides evidence of stimulated activation of the Th2 differentiation pathway from CD4⁺ naive T cells in AC mice (Fig. 3). These findings suggest that in this AC model, TSLP-activated DCs, probably through OX40L-OX40 interaction, prime the CD4⁺ T cells to differentiate into Th2 cytokine-producing cells by the activation of the IL-4-dependent transcription factors GATA-3 and STAT6.

To investigate the direct effects of epithelial cell-derived TSLP on the activation of dendritic cells, we performed an in vitro study using a human leukemia monocyte line, THP-1, that has been proven to be induced to CD11c⁺ iDCs.³⁵ Our data showed that epithelial TSLP could promote iDC maturation with significantly higher expression of DC surface markers CD80 and CD86. These results, however, were limited; further studies are needed to confirm these findings and to evaluate other effects using mouse AC model in vivo and ex vivo experiments.

In summary, this study provides new findings regarding the role of local mucosal epithelial cells in the initiation of ocular allergic inflammation through the production of a novel pro-allergic cytokine, TSLP, which activates dendritic cells to prime Th2 differentiation and allergic inflammation through TSLP-TSLPR and OX40L-OX40 signaling pathways. However, this pilot study does not provide direct evidence to confirm the functional role of TSLP in allergic inflammation. Further studies are necessary to investigate the dynamic expression of TSLP in acute and chronic allergic conjunctivitis, the direct effect of TSLP on DC activation and maturation, and the functional role of TSLP in the immunopathophysiology of ocular allergic inflammation.

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