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TSLP Expression: Analysis with a ZsGreen TSLP Reporter Mouse

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Thymic stromal lymphopoietin (TSLP) is a type I cytokine that plays a central role in induction of allergic inflammatory responses. Its principal targets have been reported to be dendritic cells and/or CD4 T cells; epithelial cells are a principal source. We report in this study the development of a reporter mouse (TSLP-ZsG) in which a ZsGreen (ZsG)-encoding construct has been inserted by recombineering into a bacterial artificial chromosome immediately at the translation initiating ATG of TSLP. The expression of ZsG by mice transgenic for the recombinant BAC appears to be a faithful surrogate for TSLP expression, particularly in keratinocytes and medullary thymic epithelial cells. Limited ZsG and TSLP mRNA was observed in bone marrow–derived mast cells, basophils, and dendritic cells. Using the TSLP-ZsG reporter mouse, we show that TNF-α and IL-4/IL-13 are potent inducers of TSLP expression by keratinocytes and that local activation of Th2 and Th1 cells induces keratinocyte TSLP expression. We suggest that the capacity of TSLP to both induce Th2 differentiation and to be induced by activated Th2 cells raises the possibility that TSLP may be involved in a positive feedback loop to enhance allergic inflammatory conditions. The Journal of Immunology, 2015, 194: 000–000.

A large body of research has implicated TSLP as playing a major role in the induction of Th2-type immune responses and in the mediation of allergic inflammation in the skin, lung, and intestine (3). There is much evidence that TSLP acts on dendritic cells (DCs) that, in turn, favor Th2 differentiation when they present Ag to naive CD4 T cells in draining lymph nodes (6, 7). In particular, TSLP-treated DCs, rather than producing proinflammatory cytokines, express OX-40 ligand, which in turn plays a role in induction of Th2 differentiation by CD4 T cells (8). Such OX-40 ligand–stimulated Th2 cells have been reported to produce substantial amounts of TNF-α and little IL-10 (6).

TSLP can also act directly on naive CD4 T cells (9) and may aid their differentiation to Th2 cells by providing the STAT5 signals that have been shown to be essential for in vitro Th2 differentiation (10). Furthermore, TSLP can synergize with IL-33 in inducing both cytokine-dependent IL-13 and IL-5 production by Th2 cells and in driving Th2 cell proliferation (11). TSLP may also enhance IL-33–mediated expansion and IL-13 production by type 2 innate lymphoid (ILC2) cells (12), potentially contributing to allergic inflammation. The relative contribution of TSLP–activated DCs, of direct action of TSLP on differentiation of naive CD4 T cells to the Th2 phenotype, and of TSLP action on differentiated Th2/ILC2 cells to sustain allergic inflammation remains to be determined.

The study of the regulation of TSLP production has been somewhat enigmatic as direct visualization of cytosolic TSLP has been difficult. In general, TSLP has been shown to be a product of epithelial cells such as skin keratinocytes (13). There is some controversy as to whether mast cells and/or basophils are a rich source of TSLP (14). It has been proposed that papain and other cysteine proteases act as allergens because they stimulate basophils to produce TSLP (15), although it is also plausible that papain acts directly on keratinocytes and other epithelial cells to induce expression of the cytokine. Strikingly, activation of PAR2 receptors has also been implicated in TSLP induction (16), although in this work it is serine proteases rather than cysteine.
proteases that are inducers. Equally interesting is the concept that TSLP may be part of a feedback loop in which it both induces/sustains IL-4/IL-13-producing Th2 cells and in which its production is stimulated by cytokines produced by inflammatory Th2 cells.

To examine these issues in greater detail, we prepared a surrogate for TSLP expression in which a ZsGreen (ZsG) construct was introduced by recombineering at the translation-initiating ATG in bacterial artificial chromosome (BAC) clone RP23-256L23. Substantial amounts of 5′ and 3′ DNA flank the TSLP gene in this 183-kb BAC, suggesting that many of the regulatory elements controlling TSLP expression may be present in the introduced genetic material, and thus that the reporter would reflect physiologic expression of TSLP.

Materials and Methods

Mice

C57BL/6 mice were purchased from Taconic Farms. BAC transgenic mice were bred, and all animals were housed, in the National Institute of Allergy and Infectious Diseases pathogen-free animal facility and used between 8 and 20 wk of age. All experiments were done under a protocol approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee.

Generation of TSLP/ZsG mice by recombineering

The murine BAC clone RP23-256L23, containing the TSLP locus, was obtained from the Children’s Hospital Research Institute BAC library (http://bacpac.chori.org). BAC DNA was purified using the Nucleobond BAC kit (Clontech) and characterized by PCR (5′-GGGACTATG-3′, 5′-GGTCTGACATG-3′). A modified BAC containing the ZsG reporter at the ATG site of the TSLP gene was done using the galK recombineering technique (17) (http://recombineering.nicrf.gov/protocol/Protocol2_CK0_vectors.pdf). Briefly, ZsG was cloned with BAC arms corresponding to the target BAC sequences surrounding the ATG at the beginning of the TSLP gene and recombineered into the BAC. The stop codon and internal polyA from ZsG were retained in a modified BAC containing the ZsG reporter at the ATG site of the MHC class II-α-alkaline phosphatase (MHC II-α), E. coli alkaline phosphatase (M-H) and ZsG+ cells were collected using a FACSaria flow cytometer (BD Biosciences) and analyzed using FlowJo Software (Tree Star, San Carlos, CA).

Preparation of thymic stromal cells for flow cytometric analysis and sorting

Thymic stromal cells were prepared using methods modified from those reported by Gray et al. (19). Following release of thymocytes by gentle teasing of the thymus, thymic fragments were digested with collagenase/ dispase at 0.25% w/v plus DNase I at 0.125% w/v (Roche) in four sequential incubations at 37°C. Reactions were stopped by addition of FCS to 20%. For thymic epithelial cell (TEC) analysis, single-cell suspensions were stained with anti–CD45.2-Pacific Blue (104; BioLegend), anti–epiluminal cell adhesion molecule (EpCAM)-PE (G8.8; eBioscience), and anti–MHC class II-α-alkaline phosphatase (MHC II-α), E. coli alkaline phosphatase (M-H) and ZsG+ cells were collected using a FACSaria flow cytometer (BD Biosciences) and analyzed using FlowJo Software (Tree Star) FACS analysis software.

RNA extraction and DNA microarray target preparation

Flow-sorted mouse cells were lysed in 600 µL RNeasy Lysis Buffer (Qiagen, Valencia, CA). Due to low number of ZsG mTEC cells (<1000), genome copy number was measured by quantitative PCR before RNA lysis was processed. Briefly, DNA was extracted from a 50-µL aliquot of cell lysate according to the Ambion DNA/RNA 96-well kit protocol. Absolute quantitation analysis of DNA used universal normalizing quantitative PCR supermix universal with premixed ROX, mouse DNA standard, and ready-made 20X mouse actB primer and probe mix according to manufacturer’s instructions (Life Technologies, Carlsbad, CA). The lowest cell copy number in the sample set was 654. Due to low number of cells, RNA lysis was combined with 12 µg linear acrylamide (Life Technologies, Carlsbad, CA) to increase RNA yield from low cell count samples. RNAs were extracted using Qiagen RNeasy 96-well system, according to manufacturer’s recommendations, except each RNA sample was treated with 27 U RNase-free DNase I (Qiagen, Valencia, CA) for 15 min at room temperature during extraction to remove genomic DNA. RNA quality was determined using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and the Agilent RNA 6000 Pico Kit RNA Kit. RNA quantitation was done by quantitative PCR using WT-Ovation Pico RNA amplification system and FL-Ovation cDNA Biotin Module, according to manufacturer’s instructions (Nugen, San Carlos, CA).

Hybridization, fluidics, and scanning were performed according to standard Affymetrix protocols (http://www.affymetrix.com). Command Console (CC v3.1, http://www.affymetrix.com) software was used to convert image files to cell intensity data (cel files). All cel files, representing individual samples, were normalized by using the trimmed mean scaling method within expression console (EC v1.2, http://www.Affymetrix.com) to produce the analyzed cel files (cph files) along with the report files. The cel files were input into Partek Genomics Suite software (Partek, St. Louis, MO; v6.6-6.12, 0907), and quantile was normalized to produce the principal components analysis (PCA) and dendrograms. NOVA was performed on the affy package. NOVA obtained multiple test corrected p values using the false discovery rate method (22) at the 0.05 significance level and was combined with fold change values, signal confidence (above background), and call consistency (as a percentage)
calculated using custom Excel templates for each comparison of interest. IPA (Ingenuity Pathway Systems, www.ingenuity.com) was used to generate a custom pathway with selected array data overlay. The array data discussed in this publication have been deposited in National Center for Biotechnology Information’s Gene Expression Omnibus (23) and are accessible through GEO series accession number GSE54343 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54343).

Confocal, two-photon intravital skin microscopy and image analysis

All tissue samples were fixed in 1% paraformaldehyde overnight at 4˚C, and then transferred into 30% sucrose overnight at 4˚C. Tissue was embedded in OCT medium (Sakura 4583), frozen on dry ice, cut with a cryostat, and kept at −80˚C until staining.

All confocal images were acquired with a Leica TCS-NT/SP confocal microscope (Leica Microsystems, Exton, PA) using a 40× oil immersion objective. Fluorochromes were excited using an argon laser at 488 nm for FITC, a krypton laser at 568 nm for Alexa 568, and a Helium-Neon laser (HeNe) at 633 nm for far-red Ab detection. DAPI was excited using an Argon laser (Enterprise model 651; Coherent) at 364 nm. Detector slits were configured to minimize any crosstalk between the channels.

Imaging of the ears by intravital multiphoton microscopy was acquired using an inverted LSM 510 NLO multiphoton microscope (Carl Zeiss Microimaging), as described previously (24). Images were processed using Leica TCS-NT/SP software (version 1.6.587), Imaris 7.5.1

FIGURE 1. TSLP-ZsG reporter mice. (A) Strategy for preparation of a BAC into which a ZsG construct with a polyA site and a stop codon has been introduced at the ATG of TSLP by recombineering. (B) Analysis of TSLP mRNA in ears of mice treated with ethanol or MC-903 for 1, 3, 4, and 5 d and a comparison of induction TSLP and CYP243A1, a known vitamin D–dependent gene, after 4 d of application of ethanol or MC-903 to the ear. This experiment was performed three times with similar results. p = 0.0055 for TSLP/18S, p = 0.006 for CYP24A1/18S. (C) Tile scans of ears of BAC transgenic mice treated with ethanol or MC-903. ZsG is shown in green, and staining with anti-cytokeratin 1 in red. (D) Ethanol was applied to the left ears and MC-903 to the right ears of ZsG BAC transgenic mice for 4 or 7 d. One day later, mice were sacrificed, single-cell suspensions from ear epidermis were prepared, and mRNA for TSLP and ZsG was determined. Mean and SEM are shown. This experiment was performed twice with similar results. (E) Regression analysis of TSLP and ZsG mRNA expression in right ears of individual mice treated with MC-903 for 4 or 7 d.
in the thymic medulla by confocal microscopy that were UEA+
was introduced by pronuclear injection into fertilized C57BL/6
ears, reaching
increases over the first 3–4 days of application of MC-903 to the
TSLP (13) and ZsG mRNA. As shown in Fig. 1B, TSLP mRNA
calcemic analog MC-903 to induce keratinocyte expression of
we used the capacity of 1,25 dihydroxy-vitamin D3 and its non-
As an initial test of the expression of ZsG as a surrogate for TSLP,
Vitamin D3 induces TSLP mRNA and ZsG expression
blotting for ZsG mRNA.
of the BAC construct, and expression was verified by Northern
thymus, TSLP was largely expressed in Hassall’s corpuscles (26). Subsequently, it was reported that, in the human thymus, TSLP was largely expressed in Hassall’s corpuscles (26). Although Hassall’s corpuscles are said to be poorly developed in the mouse, because we had the capacity to isolate TSLP-expressing thymocytes based on ZsG expression, we undertook to study these cells. We identified a small population of ZsG+ cells in the thymic medulla by confocal microscopy that were UEA+ (Fig. 2A). Flow cytometric analysis indicated that these ZsG+ cells constituted ~1% of mTECs, defined by their coexpression of EPCAM, UEA, and MHC class II (27, 28) (Fig. 2B). We purified cTECs (EPCAM+, UEA− cells) and mTECs (EPCAM−, UEA+ cells); the latter were separated into ZsG+ and ZsG− populations. cTECs expressed >40 times more β5T mRNA than the ZsG− or ZsG+ mTECs (Fig. 3). β5T is a thymoproteasome subunit report- ed to be exclusively expressed in cTECs (29). ZsG− mTECs expressed >14 times as much autoimmune regulator (AIRE) mRNA than the cTECs. These relative expression results confirm our purification procedure. The ZsG+ and negative mTECs expressed similar amounts of AIRE mRNA. Essentially all ZsG mRNA was expressed in the ZsG+ mTECs, and TSLP mRNA was expressed to a substantially greater degree in the ZsG+ mTECs compared with ZsG− mTECs. In a subsequent analysis of mRNA expression, we observed the involucrin, a marker for terminal epidermal differentiation (30), was ~4-fold overexpressed in ZsG+ mTECs compared with ZsG− cells (Fig. 3).

To take further advantage of the ZsG marker, we purified both cTECs and ZsG+ and negative mTECs and carried out a micro-array analysis. Strikingly, principal component analysis revealed that ZsG+ mTECs were substantially different from ZsG− mTECs and from cTECs (Supplemental Fig. 1). Interestingly, ATG13, BCN1, and Eph receptor A1 were overexpressed in ZsG+ mTECs;
ATG13 has 6.8-fold ratio in ZsG+ compared with ZsG− mTECs, BCN1, 14.9, and Eph receptor A1 3.2. All three are part of an autophagy pathway that is regulated by mTOR and that, in other situations, may be involved with autophagic cell death. Indeed, as shown in Supplemental Fig. 2, ATG13, BCN1, and EPH A1 are part of a pathway in which several of the members are overexpressed and in which other members of the pathway that are normally inhibited when ATG13 and related genes are overexpressed are, in fact, downregulated (31). In prostatic epithelial cells and endothelial cells, but not fibroblasts, treatment with ephrin-A1 inhibits cell proliferation (32).

TNF-α and IL-4/13 induce ZsG expression in keratinocytes

We then turned to the analysis of ZsG expression in the back skin or ears of mice in response to injection of various cytokines. PBS, TNF-α (1 μg), IL-13 (1 μg), or IL-4 (1 μg) was injected into the shaved back skin of TSLP-ZsG transgenic mice on successive 2 d. Confocal microscopy carried out on skin biopsies taken 24 h later revealed that TNF-α, IL-4, and IL-13 each caused induction of ZsG, with TNF-α being the most striking inducer (Fig. 4A). A particularly graphic example of the degree of induction is shown by injection of TNF-α or PBS into the ear of TSLP-ZsG mice twice and examination by multiphoton microscopy 24 h later (Fig. 4B). In this case, the keratinocytes are being seen from directly above, whereas in the confocal views, they are being seen transversely.

We also evaluated ZsG expression in response to TNF-α by flow cytometry. Staining epidermal preparations from injected skin with anti-CD3, anti-CD11c, and anti-cytokeratins 10 or 14 revealed three major cell populations. Because staining with anti-cytokeratins required fixation, which diminishes ZsG fluorescence, we repeated the staining on nonfixed cells with anti-CD3 and anti-CD11c only. Only the cells negative for both these markers expressed ZsG, implying that only the anti-cytokeratin-positive cells expressed ZsG (Fig. 5). Because cytokeratins are mainly restricted to epidermal cells (33), we conclude in mice stimulated with TNF-α, ZsG expression in the skin was confined to the keratinocyte population.

In addition to TNF-α, IL-4 and IL-13, IL-1, and LPS each induced ZsG expression upon injection into back skin when evaluated by confocal microscopy (data not shown).

Subcutaneous activation of Th2 and Th1 cells induces ZsG expression

The finding that TNF-α and IL-4/13 induce TSLP/ZsG expression when injected into the skin implies that CD4 T cells capable of making these cytokines, if stimulated in the skin, should induce keratinocytes to express ZsG. Accordingly, we primed OT-2 cells in vitro under Th1- or Th2-inducing conditions through one or two rounds of priming. We verified that the cells had attained either a Th1 or a Th2 phenotype based on their production of IFN-γ or of IL-4/13 (Fig. 6A). Both the Th1 and Th2 cells were robust producers of TNF-α. After culturing the primed cells in IL-2 (Th1) or IL-2 and IL-7 (Th2), we transferred 3–5 × 10^6 cells i.v. Twenty-four hours later, we challenged the mice with 100–250 μg OVA or, as a control, with PBS, in the back on successive 2 d and evaluated keratinocyte expression of ZsG 24 h later by confocal microscopy. Recipients of both Th1 and Th2 cells showed strong induction of ZsG expression in the keratinocyte layer in response to challenge with OVA, but not hen egg lysozyme (Fig. 6B). PBS-injected controls showed little or no induction.

We also tested the contribution of IL-4/IL-13 to the Th2 induction of ZsG expression by keratinocytes. Th2 cells were transferred into B6 mice. These animals were challenged with lysozyme or OVA. Some of the OVA-treated mice received an i.v. injection of monoclonal anti-IL-4Rα (M1) prior to challenge. This...
treatment reduced, but did not abolish ZsG induction, implying that IL-4 or IL-13 was responsible for only some of the induction of ZsG and presumably TSLP by Th2 cells (Fig. 7).

**TSLP/ZsG expression in bone marrow–derived DCs, basophils, and mast cells**

Although there is general agreement that epithelial cells can express TSLP when appropriately stimulated, there has been controversy about expression of TSLP by mast cells, basophils, and DCs (14). To study this point, we prepared bone marrow–derived mast cells, basophils, and DCs by culturing bone marrow cells from TSLP-ZsG and wild-type B6 mice in IL-3 (mast cells and basophils) or GM-CSF (DCs). On day 9 of the IL-3 culture, both FcεRI+ c-kit+ cells, identified as mast cells (34), and FcεRI+, c-kit+, and CD49+ cells, identified as basophils, were present (35). When analyzed by flow cytometry, we found few, if any, mast cells or basophils to be ZsG+ after stimulation with PMA and ionomycin (Fig. 8). We analyzed DCs on day 8 of culture; no ZsG+ cells were seen by flow cytometry (data not shown).

Nonetheless, when we analyzed message expression, we did detect induction of both TSLP and ZsG mRNA in bone marrow–derived mast cells, basophils, and DCs (Fig. 8). Basophils and mast cells were stimulated with PMA and ionomycin and analyzed 3 h later; DCs were stimulated with LPS plus IL-4 for 4 h. Based on our failure to detect ZsG protein expression in mast cells, basophils, or DCs, we conclude that these three cell types, although capable of producing TSLP, do so to a much lower extent than do keratinocytes. Consistent with this, when we compared cycle number differences in the real-time PCR analysis for TSLP, ZsG, and 18S RNA, MC-903–stimulated keratinocytes showed an apparent greater expression of 100- to >1000-fold (Supplemental Fig. 3) than did the three hematopoietic cell types. Even if 18S RNA expression is somewhat different in these various cell types, this result is nonetheless consistent with a very much lower degree of expression of both TSLP and ZsG in DCs, basophils, and mast cells than in keratinocytes.

**Discussion**

We provide evidence that green fluorescence by cells from a BAC-transgenic mouse in which ZsG has been recombineered into the TSLP gene of BAC RP23-256L23 at the translation initiating ATG is a good reporter of TSLP expression. Vitamin D3 and MC903 were strong stimulants of ZsG expression. They modify retinoid X receptor nuclear factor regulation of the TSLP promoter (36). TNF-α was also a very strong stimulant of ZsG expression, presumably acting through TNF-RI expressed on keratinocytes and activating NF-κB and AP-1 (37). The mechanisms through which IL-4 and IL-13 induce TSLP expression are not clear.

Keratinocytes showed bright fluorescence upon stimulation by these stimulants injected into the ear or the skin of the back; LPS and IL-1β also modestly induced ZsG expression. To date, we have observed little or no fluorescence in hematopoietic cells at sites of vitamin D3 or cytokine injection.

TSLP has been reported to exert much of its function by acting on DCs in the tissues and causing them to adopt a phenotype that was most effective in stimulating responding CD4 T cells to differentiate to Th2 cells (6, 7). The TSLP-treated DCs express OX-40 ligand and fail to make cytokines such as IL-12 that are
associated with differentiation of responding CD4 T cells to a Th1 fate (8). The expression of OX-40L on DC aids their priming of normal Ag-specific naive CD4 T cells to Th2 cells. Furthermore, Liu and colleagues (6) have observed that CD4 T cells primed by OX-40L–expressing APC are more likely to produce TNF-α and less likely to secrete IL-10, suggesting that they may be regarded as inflammatory Th2 cells.

However, there are at least two other options for the function of TSLP in controlling allergic inflammation. Naive CD4 T cells express functional TSLP receptors and thus can transduce a STAT5 signal when stimulated with TSLP (5). In vitro Th2 differentiation is very dependent on STAT5 activation (10). TSLP should be capable of replacing IL-2 in such priming, and thus, production of TSLP in the vicinity of Ag-priming of naive CD4 T cells could provide the STAT5 signal needed for Th2 differentiation. However, such a scenario would require a reliable source of TSLP at or near the site of priming. Naive CD4 T cell priming occurs in the paracortex of the lymph node or in the comparable region of the spleen where little or no TSLP would be expected to be present except, possibly, as reported by Sokol et al. (15), as a result of the transient recruitment of basophils to the lymph node in papain immunization and the stimulation of these cells to produce TSLP. We also showed that Ag-mediated activation of Th2 or Th1 cells in the skin resulted in robust induction of ZsG in the keratinocytes, indicating that in situ Ag-driven CD4 T cell activation drives TSLP induction, presumably through production of TNF-α, IL-4, or IL-13 production. The mutual stimulatory properties of keratinocyte products (TSLP and IL-33) and Th2 (or possibly Th1) cell products (IL-13 in particular) could result in a chronic Ag-independent Th2-mediated allergic inflammatory response. A similar mechanism might hold for ILC2 cells.

An alternate possibility is that memory/effector Th2 cells would be attracted to tissues into which Ag has been introduced. If TSLP had been induced at such sites, such as might be the case in atopic dermatitis (38), then such TSLP could act on differentiated Th2 cells, possibly together with IL-33, to drive IL-13 and IL-5 production and to enhance cell proliferation (11). An even more intriguing possibility is that Ag stimulation of Th2 cells in the tissues, particularly Th2 cells that produce TNF-α, as has been reported to be the case for CD4 cells induced by TSLP-treated DCs (6), would induce epithelial cells to produce TSLP. Indeed, we observed in this study that TNF-α, IL-4, and IL-13 induce keratinocytes from reporter mice to become ZsG+, and others have previously reported that these cytokines can cause TSLP production by keratinocytes (39–41). We also showed that Ag-mediated activation of Th2 or Th1 cells in the skin resulted in robust induction of ZsG in the keratinocytes, indicating that in situ Ag-driven CD4 T cell activation drives TSLP induction, presumably through production of TNF-α, IL-4, or IL-13 production. The mutual stimulatory properties of keratinocyte products (TSLP and IL-33) and Th2 (or possibly Th1) cell products (IL-13 in particular) could result in a chronic Ag-independent Th2-mediated allergic inflammatory response. A similar mechanism might hold for ILC2 cells. These cells have been reported to show enhanced IL-13 production when stimulated with IL-33 and TSLP, and the IL-13 they produce could contribute to a chronic allergic inflammatory response (12).

**FIGURE 6.** Stimulated Th1 and Th2 cells induce ZsG expression. (A) Th1 and Th2 cells were prepared from OT-II cells, and their expression of IL-4, IFN-γ, and TNF-α upon stimulation with PMA and ionomycin is indicated. (B) Confocal microscopy of back skin of mice that received Th1 or Th2 OT-II cells (see Results) and was challenged by injection of lysozyme or OVA. This experiment was performed three times with similar results. (C) Flow cytometry of epithelial cells from the back skin of mice that received Th1 or Th2 OT-II cells and was challenged with PBS or with 50 or 250 μg OVA. This experiment was performed twice with similar results.
We were surprised by our failure to observe ZsG+ hematopoietic cells in skin or ear as well as in IL-3–driven bone marrow cell cultures and in GM-CSF–induced DCs. Indeed, Kashyap et al. (42) have recently reported that lung DCs from mice challenged with house dust mites expressed TSLP mRNA. The failure of IL-3–driven mast cells and basophils and of GM-CSF–induced DC to be ZsG+ on flow cytometry or confocal microscopy may reflect the presence of relatively small amounts of TSLP and ZsG in these cells compared with that in stimulated keratinocytes. However, in view of our failure to detect ZsG in stimulated basophils and/or mast cells, one may ask whether these cells are physiologically important sources of TSLP. These cells could have a different role in TSLP expression, such as through the release of proteases or proinflammatory cytokines that induce TSLP production by epithelial/stromal cells.

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Disclosures

The authors have no financial conflicts of interest.

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