Thymic Stromal Lymphopoietin Interferes with Airway Tolerance by Suppressing the Generation of Antigen-Specific Regulatory T Cells

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Thymic Stromal Lymphopoietin Interferes with Airway Tolerance by Suppressing the Generation of Antigen-Specific Regulatory T Cells

Liying Lei,* Yanlu Zhang,*,† Weiguo Yao,* Mark H. Kaplan,*‡ and Baohua Zhou*,‡

Thymic stromal lymphopoietin (TSLP) is an essential cytokine for the initiation and development of allergic inflammation. In this study, we have investigated the role of TSLP in the breakdown of immune tolerance and generation of inducible regulatory T cells (iTregs). Our results demonstrated that TSLP diverted airway tolerance against OVA to Th2 sensitization and inhibited the generation of OVA-specific iTregs. TSLP exerted a direct inhibitory effect on both human and mouse iTreg development in vitro. Low doses of TSLP were capable of inhibiting iTreg induction without significantly promoting Th2 development, indicating that these two functions of TSLP are separable. Moreover, the TSLP-mediated inhibition of iTreg generation was only partially dependent on IL-4 and Stat6, and was effective when TSLP was present for the first 24 h of T cell activation. These results define a novel role for TSLP in regulating the balance of airway tolerance and allergic inflammation.

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Abbreviations used in this article: DC, dendritic cell; eGFP, enhanced GFP; i.n., intranasal; iTreg, inducible regulatory T cell; PAS, periodic acid-Schiff; Treg, regulatory T cell; TSLP, thymic stromal lymphopoietin.

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100 μg OVA with 500 ng TSLP in 40 μl PBS. As controls, mice received PBS or TSLP. Mice were challenged i.n. with 50 μg OVA in PBS for 3 consecutive days from day 14. Airway hyperresponsiveness (24) in these mice was tested 24 h after the last challenge. Enhanced pause in response to increasing doses of aerosolized methacholine (Sigma-Aldrich) in PBS was analyzed using unrestrained whole body plethysmography (Buxco Electronics).

Bronchoalveolar lavage, tissue fixation, and staining

After the mice were euthanized, lungs were lavaged three times with 1 ml each warm PBS via a tracheal polyethylene catheter. Total cells in bronchoalveolar lavage fluid were counted with a hemocytometer, whereas differential cell counts were performed using cytopsin (Shandon) cell preparations stained with modified Wright-Giemsa stain.

After lavage, lungs were excised from thorax cavity. The lungs were inflated with 4% neutral buffered formaldehyde (Fisher Biotech) and fixed overnight at room temperature. Tissues were then embedded in paraffin, sectioned, and stained with H&E or periodic acid-Schiff (PAS) stain.

Quantitative RT-PCR

RNA from lungs or cell cultures was extracted using TRIzol (Invitrogen), according to the manufacturer’s protocol, and reverse transcribed to cDNA using the high capacity cDNA reverse-transcription kit (Applied Biosystems). Quantitative RT-PCR was performed using TaqMan chemistry on a 7500 Fast Real-Time PCR System (Applied Biosystems). TaqMan primer and probe mixtures were purchased from Applied Biosystems. GAPDH was used as endogenous control reference gene to normalize sample variation, and relative expression was calculated by the change-in-threshold (−ΔΔC_T) method.

Naive T cell isolation, culture, and in vitro Treg generation

Naive mouse CD4^+CD62L^+ T cells were isolated from spleens and lymph nodes, and naive human CD4^+ cells were isolated from PBMC using MACS isolation kit (Miltenyi Biotec). RPMI 1640 medium was supplemented with 10% FBS, 1 mM sodium pyruvate, 10 mM HEPES buffer, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME. Effects of TSLP on iTreg differentiation in vitro were studied using either a standard

FIGURE 1. TSLP diverts airway tolerance to Th2 sensitization against innocuous Ag OVA. BALB/c mice were conditioned i.n. with PBS, TSLP, OVA, or OVA + TSLP for 3 d. Ten days later, the animals were challenged i.n. with OVA three times. A, Airway responsiveness to increasing dose of methacholine was analyzed by unrestrained whole body plethysmography and is presented as average enhanced pause (Penh) over a 3-min period. B, Total cell and eosinophil count in bronchoalveolar lavage fluid. C, Quantitative PCR analysis of the expression of Th2 cytokines IL-4, IL-5, and IL-13 in the lungs, presented relative to the expression in PBS-conditioned and OVA-challenged mice. D, Lung tissue sections stained with H&E showing peribronchiolar inflammatory infiltration in OVA + TSLP–conditioned mice after challenge. E, Quantitative PCR analysis of the expression of Fizz1 gene in the lungs, presented relative to the expression in PBS-conditioned and OVA-challenged mice. F, Lung tissue sections stained with PAS showing goblet cell hyperplasia/metaplasia in OVA + TSLP–conditioned mice after challenge. G, Quantitative PCR analysis of the expression of mucus gene Muc5ac, presented relative to PBS-conditioned and OVA-challenged mice. Original magnification ×10 (D, F, P). PBS-conditioned mice; O, OVA-conditioned mice; T, TSLP-conditioned mice; T + O, OVA + TSLP–conditioned mice. Data shown represent mean ± SEM (n = 4) from one of two independent experiments. **p < 0.01; ***p < 0.001. ns, not significant by ANOVA with Bonferroni’s post hoc tests.
protocol (25) or a protocol without exogenous TGF-β1 modified from Sauer et al. and Walker et al. (26, 27). Briefly, in the standard protocol, naïve T cells were cultured for 5 d in the presence of 10 μg/ml plate-bound anti-CD3 (17A2; Biolegend), 2 μg/ml soluble anti-CD28 mAb (37.51; Biolegend), and 3 ng/ml TGF-β1 (PeproTech). For the protocol without exogenous TGF-β1, naïve T cells were activated with 5 μg/ml plate-bound anti-CD3 and 1 μg/ml anti-CD28 without TGF-β for 24 h. On the second day, cells were removed from activation stimuli, washed, and cultured for 9 more days. For selected treatments, rTSLP (R&D Systems) was added to culture, as indicated.

Adoptive transfer experiments

Naive CD4+CD62L+ T cells were isolated from DO11.10 mice, and adoptively transferred into wild-type or TSLPR-deficient recipient mice via tail vein injection at 3–4 × 10^6 T cells in 200 μl PBS. Twenty-four hours after cell transfer, mice were treated i.n. with 100 μg OVA or 100 μg OVA plus 500 ng TSLP for 3 or 5 d.

To examine the effect of TSLP on Ag-specific Treg generation in an Ag-driven asthma model, groups of wild-type BALB/c mice were sensitized to OVA by i.p. injection of 50 μg OVA emulsified in 1.3 mg aluminum hydroxide on days 0 and 7. On day 13, 3 × 10^6 purified wild-type or TSLPR-deficient CD4+ naïve T cells were i.v. injected into these sensitized mice. Starting from day 14, the mice were challenged with 25 μg OVA for 5 d.

To track OVA-specific iTreg generation, lung-draining mediastinal lymph nodes were harvested from the recipient mice. Cells were stained with CD4 and KJ1-26 Abs, followed by intracellular staining for Foxp3 (Biolegend).

Flow cytometry, Abs, and reagents

The following mAbs were purchased from Biolegend unless stated otherwise: CD4 (clone GK1.5), DO11.10 TCR (clone KJ1-26), Foxp3 (clone 150D), IL-4 (clone 11B11), and IFN-γ (clone XMG1.2). Rat IgG1 (clone RTK2071), rat IgG2a (clone RTK2758), and rat IgG2b (RTK4530) were included as isotype controls. All surface and Foxp3 staining was carried out according to manufacturer’s protocol. To detect intracellular cytokine production, cells were restimulated with 50 ng/ml PMA and 500 ng/ml ionomycin for 6 h. Monensin (Biolegend) was added to the culture for the last 4-h incubation. Cells were then stained for IL-4 and IFN-γ production, as described (24).

Results

TSLP breaks immune tolerance while promoting Th2 sensitization in vivo

Previous studies showed that TSLP is strongly associated with respiratory and dermal allergic inflammatory diseases (7, 9–11, 28, 29). However, repeated i.n. instillation of TSLP did not induce airway inflammation, but rather predisposed airway mucosa toward the development of aberrant responses against innocuous environmental Ags (30). To determine whether TSLP can break airway tolerance toward harmless Ags like OVA, we examined the effect of TSLP at primary immunization when a tolerance-vs-immunity decision was made in the airway mucosa. BALB/c mice were subjected to i.n. TSLP, administered in the presence or absence of OVA, for consecutive 3 d. Control groups received OVA or PBS. After 10 d, these mice were challenged three times to examine the airway response against OVA. OVA plus TSLP-conditioned mice developed strong airway hyperresponsiveness (Fig. 1 A) and airway eosinophilia (Fig. 1B), whereas OVA-conditioned mice were tolerant to OVA challenge. Neither TSLP- nor PBS-conditioned mice displayed pulmonary inflammation. In addition, lungs from OVA plus TSLP-conditioned mice showed a Th2-biased cytokine environment with high IL4,
II5, and II13 expression in the lungs (Fig. 1C). Histopathology analysis demonstrated that only OVA plus TSLP–conditioned mice developed allergic airway inflammation (Fig. 1D), which was confirmed by significantly increased expression of Fizz1 (Fig. 1E), a gene associated with pulmonary inflammation. On PAS-stained slides, the magenta-colored mucus-producing cells were

**FIGURE 3.** Effects of TSLP on iTreg generation in vitro. A, TSLP suppressed murine iTreg differentiation in the presence of TGF-β1. Naive CD4+ CD62L+ T cells were isolated from spleens and lymph nodes, cultured with 10 μg/ml plate-bound anti-CD3 and 2 μg/ml anti-CD28 in the presence of 3 ng/ml TGF-β1 for 5 d. TSLP was used at 10 ng/ml. Data represent mean ± SEM (n = 3). B, TSLP suppressed human iTreg differentiation in the presence of TGF-β1. Naive CD4+ T cells were isolated from PBMC and cultured in the same condition as A. Human T cells showed considerable variability in responding to TSLP. A responder is shown on top, and a nonresponder on the bottom. The percentage inhibition of iTreg differentiation by TSLP from all 20 donor samples is indicated in the graph on the right. C, Purity of naive CD4+ T cells isolated from human PBMC. Samples of PBMC and purified T cells from the same donor were stained with Blood Dendritic Cell Enumeration Kit (Miltenyi Biotec) and analyzed by flow cytometry. Data represent mean ± SEM (n = 6). D, Quantitative PCR analysis of the expression of human TSLPR in activated CD4+ T cells, presented relative to naive cells. Inclusion of TSLP in culture (+TSLP) further enhanced TSLPR expression. Naive CD4+ T cells were purified from PBMC and cultured in the same condition as A. Data represent mean ± SEM (n = 6). *p < 0.05, ***p < 0.001, one-way ANOVA.

**FIGURE 4.** Potency of TSLP in inhibiting iTreg generation. A, TSLP suppressed murine iTreg differentiation in a dose-dependent manner. Naive CD4+ T cells isolated from spleens and lymph nodes of Foxp3-eGFP mice were cultured with 5 μg/ml plate-bound anti-CD3 and 1 μg/ml anti-CD28 for 24 h, removed from stimuli, and cultured for another 9 d for Treg generation. TSLP was included in the culture at concentrations as indicated throughout 10 d of culture. Data represent mean ± SEM (n = 3) from one of three independent experiments. B, Both percentage and number of Foxp3-eGFP+ cells were reduced in response to increasing dose of TSLP. C, Specificity of TSLP in suppressing iTreg differentiation. Naive CD4+ T cells were isolated from TSLPR-deficient mice and cultured in the conditions as described in A. No significant inhibition of iTreg differentiation was seen even when 20 ng/ml TSLP was included in the culture.
rarely seen in PBS-, TSLP-, and OVA-conditioned mice, but were abundant in OVA plus TSLP-conditioned mice, indicating goblet cell hyperplasia/metaplasia and increased mucus production (Fig. 1F). Consistent with the PAS staining, expression of mucus gene Muc5ac in the lungs was significantly elevated in OVA plus TSLP-conditioned mice (Fig. 1G). Therefore, i.n. exposure to OVA renders wild-type BALB/c mice tolerant to OVA challenge, whereas the presence of TSLP during primary immune response is sufficient to divert this tolerance to Th2 sensitization, leading to strong allergic airway inflammation with all the cardinal features of asthma following OVA challenge.

**TSLP inhibits Ag-specific iTreg development in vivo**

It is well established that TSLP is capable of promoting Th2 differentiation (2). To determine whether elevated TSLP can suppress the development of airway mucosal tolerance, we examined the capacity of TSLP to inhibit Ag-specific iTreg generation in an in vivo system. BALB/c wild-type or TSLPR-deficient mice were transferred with naïve CD4+CD62L+ DO11.10 TCR transgenic T cells i.v., and 24 h later were treated i.n. with OVA in the presence or absence of TSLP daily for 3 or 5 d. Expression of Foxp3 in donor OVA-specific KJ1.26+ T cells from lung-draining mediastinal lymph nodes was analyzed using flow cytometry (Fig. 2A). Mice that received OVA plus TSLP treatment had significantly lower percentages of OVA-specific iTregs in their mediastinal lymph nodes, compared with OVA-only–treated mice (Fig. 2B). Whereas Ag-specific iTregs continued to accumulate in the lung-draining lymph nodes of mice from day 3 to day 5, such increase of OVA-specific iTregs was completely prevented by TSLP (Fig. 2B). These results indicate that TSLP can antagonize Ag-induced airway tolerance through suppressing Ag-specific iTreg generation. More strikingly, TSLP was equally efficient in suppressing OVA-specific iTreg accumulation in mediastinal lymph nodes of TSLPR-deficient recipient mice (Fig. 2C). The fact that only donor DO11.10 T cells were able to respond to TSLP suggests that TSLP directly acts on CD4+ T cell to suppress their conversion to iTregs, although we cannot rule out the possibility that TSLP can also affect other cell types such as DCs to indirectly suppress iTreg development in vivo.

It has been reported that LPS induced OX40L expression on DCs and B cells that resulted in a synergistic activity between TLR4 and OX40 signals, leading to production of IL-4, IFN-γ, and IL-6, which blocked Treg development (31). To rule out the possibility that contaminants in the rTSLP played a similar role, we assessed the role of TSLP in inhibiting Ag-specific iTreg differentiation in vitro. Naïve CD4+CD62L+ T cells were isolated from mouse spleens and lymph nodes, and stimulated with plate-bound anti-CD3, soluble anti-CD28, and TGF-β. After culture for 5 d, ~30% of murine CD4+ T cells differentiated to iTregs, as indicated by intracellular staining for Foxp3. In the presence of 10 ng/ml TSLP, the CD4+Foxp3+ T cell population was significantly reduced (Fig. 3A).

TSLP exhibited inhibitory effects on human iTreg generation in vitro from naïve CD4+ T cells isolated from some donors, but not all subjects (Fig. 3B). Naïve CD4+ T cells obtained from anonymous donors showed a wide range of susceptibility to TSLP-mediated iTreg differentiation. TSLP had no effects on some donors, while inhibiting >70% iTreg differentiation in others (Fig. 3B). We are currently investigating whether the response of human CD4+ T cells to TSLP is related to predisposition.

**TSLP inhibits the generation of iTregs in vitro**

To further assess the ability of TSLP to directly act on CD4+ T cells, we analyzed the effect of TSLP on mouse and human iTreg differentiation in vitro. Naïve CD4+CD62L+ T cells were isolated from mouse spleens and lymph nodes, and stimulated with TSLP with reduced efficiency. Naïve CD4+ T cells were isolated from Foxp3Fgf mice and cultured, as described in Fig. 3C. After the culture, cells were restimulated and stained for intracellular IL-4 production. iTreg generation (eGFP expression) and Th2 differentiation (IL-4 expression) of the cultured cells were examined by flow cytometry. B. Comparison of dose-response curves of TSLP in inhibiting iTreg generation and in promoting Th2 differentiation. Data represent mean ± SEM (n = 3) from one of three independent experiments. C. TSLP suppressed iTreg induction from IL-4− and Stat6−deficient naïve CD4+ T cells with reduced efficiency. Naïve CD4+ T cells were isolated from wild-type, Il4−/−, and Stat6−/− mice, and cultured as described in Fig. 4A.
tion to allergic diseases. Because it is reported that human TSLP mainly exerts its effect through DCs and had little effect on CD4+ T cells (32), we examined DC contamination in our purified human naive CD4+ T cells by Blood Dendritic Cell Enumeration Kit (Miltenyi Biotec). Compared with PBMC, which contained 0.34% (± 0.21, n = 6) myeloid DCs and 0.42% (± 0.1) plasmacytoid DCs, our purified naive human CD4+ T cells contained only 0.037% (± 0.026, n = 6) myeloid DCs and 0.024% (± 0.046) plasmacytoid DCs, not significantly different from isotype-stained PBMC (Fig. 3C). These results suggest that TSLP is acting directly on human T cells, and not indirectly through DCs. Consistent with other reported results (32–34), TSLPR expression was increased following T cell activation and was further enhanced when 10 ng/ml TSLP was included in the culture (Fig. 3D).

Potency of TSLP in inhibiting iTreg generation in vitro

Similar to other cytokines antagonizing iTreg induction (35), TSLP was more potent in suppressing iTreg differentiation when TGF-β levels were low. To eliminate the interference from TGF-β, we adapted an iTreg differentiation protocol without adding TGF-β based upon two published studies (26, 27). Purified CD4+CD62L+ T cells were activated with plate-bound anti-CD3 and soluble anti-CD28 for 24 h. Following activation, cells were removed from TCR stimulation, washed, and cultured for additional 9 d. Compared with the standard protocol in which TCR stimulation was present during the entire 5-d culture, the 24-h activation more closely mimics the in vivo scenario, because it has been reported that interaction of DCs with naive Ag-specific CD4+ T cells occurred within 18–24 h after immunization, followed by resumed movement and dispersion of activated CD4+ T cells, in both tolerizing and priming conditions (36, 37).

Using Foxp3egfp knockin mice as the source of naive CD4+ T cells, we found that after 10-d culture 17.1% cells differentiated into iTregs, as identified by enhanced GFP (eGFP) expression. Inclusion of TSLP in the culture inhibited the induction of iTreg in a dose-dependent manner with 3.45% eGFP-positive cells at 10 ng/ml TSLP (Fig. 4A, 4B). In this culture condition without adding TGF-β, iTreg differentiation is very sensitive to TSLP because as low as 0.05 ng/ml TSLP was enough to result in a 50% reduction in Foxp3egfp+ cells. The number of Foxp3egfp+ generated in these cultures was also reduced in a dose-dependent manner (Fig. 4B). Such inhibitory effect is TSLP specific, as naive CD4+ T cells isolated from TSLPR-deficient mice showed normal iTreg induction even in the presence of 20 ng/ml TSLP (Fig. 4C).

TSLP can inhibit iTreg induction independent of IL-4 and Stat6

Studies have shown that transgenic expression of TSLP in skin and lung induced strong Th2 inflammation. In vitro experiments demonstrated that TSLP-treated DCs promoted naive T cells to differentiate into Th2 lineage and produce Th2 cytokines, including IL-4, IL-5, and IL-13 (3, 12). TSLP could also directly exert its effect on CD4+ T cells to enhance their IL-4 and IL-13 secretion without the help of DCs in vivo and in vitro (11, 13). Indeed, in our culture system, TSLP induced IL-4+ Th2 cell differentiation in a dose-dependent manner (Fig. 5A). Th differentiation cytokines IL-4, IL-6, IL-12, and IFN-γ have been demonstrated to suppress the generation of Foxp3-expressing iTregs (35, 38). However, no significant increase of IL-4–producing cells was observed when 1 ng/ml TSLP was included in the culture (Fig. 5A). Thus, TSLP appeared to be more efficient in inhibiting iTreg generation than in promoting Th2 polarization. We superimposed the TSLP dose–response curves in inhibiting iTreg generation and in promoting Th2 differentiation (Fig. 5B). As low as 0.05 ng/ml TSLP was sufficient to induce a 50% reduction in Foxp3+ iTreg population, whereas >1 ng/ml TSLP was needed to induce significant IL-4+ Th2 population in the same culture.

To further analyze whether TSLP was able to inhibit iTreg induction independent of its Th2-promoting effects, we cultured naive CD4+ T cells isolated from Il4- or Stat6-deficient mice with or without TSLP. The Foxp3+ iTreg population in wild-type CD4+ T cell culture decreased from 14.3% to below 3% in the presence of 10–20 ng/ml TSLP. In both Il4−/− and Stat6−/− CD4+ T cell cultures, iTreg population decreased from 16% to ~8% in the presence of TSLP (Fig. 5C). Thus, TSLP was still capable of inhibiting Treg generation in the absence of IL-4 or Stat6, although the efficiency was decreased. Taken together, these data indicate that TSLP is able to suppress iTreg induction even at low levels without inducing significant Th2 differentiation. With increasing concentrations, TSLP can further dampen iTreg generation through promoting Th2 polarization.

Presence of TSLP during T cell culture is sufficient to inhibit iTreg generation

Studies indicated that T cell activation in vivo is likely to occur within 24 h after Ag administration (36, 37). We next investigated
whether TSLP is required throughout the 10-d culture period or only during the 24-h T cell activation phase. We added TSLP to culture at various time points during and/or after activation and performed FACS analysis after 10 d of culture for Foxp3 expression. As shown in Fig. 6A, the presence of TSLP during TCR stimulation on day 1 was sufficient to suppress Foxp3 expression in those T cells. The inhibitory effect was as efficient as the presence of TSLP throughout the 10-d culture. Adding TSLP after TCR activation from day 2 to day 10 resulted in a much weaker suppression on iTreg generation (Fig. 6B). It is known that TSLP promotes both human and mouse CD4+ T cell proliferation and survival (14, 32, 33, 39). We found that cultures with TSLP throughout the 10-d culture and from day 2 to day 10 generated significantly more Foxp3+GFP+ cells than cultures with TSLP only on day 1 (Fig. 6B). These data suggest that TSLP present after CD4+ T cell activation promotes T cell survival and/or proliferation without discriminating Tregs or effector T cells. Therefore, it is critical for TSLP to be present at the time of T cell activation to inhibit iTreg generation.

**Discussion**

Development of tolerance against environmental allergens protects individuals from developing pathological responses, and failures in the mechanisms of tolerance lead to the sensitization to allergens and to inflammatory reactions such as allergic asthma. The induction of adaptive Foxp3+ iTregs is essential to establish mucosal tolerance by the oral and respiratory routes (20, 21). We report in this study a novel function of TSLP on inhibiting iTreg induction. It is well established that TSLP promotes Th2 differentiation (2). The fact that low concentration of TSLP significantly inhibits iTreg induction without promoting Th2 development presents a new mechanism for TSLP to regulate the balance of airway tolerance and allergic inflammation.

It is well established that TSLP is a proallergic factor to promote Th2 differentiation by activating DCs (2). Recently, TSLP was also shown to positively affect Treg generation indirectly through its action on DCs (15–19). Such seemingly contradictory results most likely stem from tissue specificity of TSLP action. For example, TSLP-activated DCs in thymus (15–17) and intestine (19) positively select Tregs. We report in this study that TSLP directly suppresses both human and mouse iTreg induction, which is separable from its ability to promote Th2 differentiation (Fig. 5). Our findings are of clinical significance because it has been shown that allergic asthma patients exhibit decreased frequency and diminished suppressive activity of pulmonary Tregs (40). Elevated TSLP in the bronchoalveolar lavage fluid from allergic asthmatic subjects inhibited IL-10 production and suppressive activity of pulmonary Tregs isolated from healthy controls (5). As we showed in this study that human TSLP inhibited iTreg differentiation (Fig. 3B), the increased pulmonary TSLP in allergic asthmatics could contribute to diminished pulmonary Tregs in these patients.

Recent animal studies suggest that TSLP might be the underlying factor to drive the "atopic march," the progression from atopic dermatitis to asthma (41, 42). The keratinocyte-derived systemic TSLP in these mice, but not the skin lesions, was shown to be responsible for augmenting allergic airway inflammation after the mice were sensitized with OVA and Alum and i.n. challenged with OVA (41, 42). Such augmentation might be attributed to the ability of TSLP to promote Th2 response. However, it was unclear how elevated TSLP at the time of sensitization still aggravated allergic airway inflammation and airway hyperresponsiveness in mice challenged 50 d later when TSLP levels were returned to normal (42). Our finding that TSLP even in low amounts suppressed iTreg induction during sensitization offers a possible explanation. Along with inducing Th2 priming, OVA plus Alum sensitization also induced Ag-specific iTregs (20, 43). The Ag-specific iTregs accumulated in the inflamed lung after OVA challenge and helped to control the severity of inflammation (20). Elevated serum TSLP ranging from 50 pg/ml to 1.5 ng/ml was detected in the mice with severe atopic dermatitis (41, 42). Although TSLP might not be necessary for promoting Th2 priming because these mice were sensitized with the strong Th2-polarizing adjuvant Alum, elevated serum TSLP at sensitization would suppress the induction of Ag-specific iTregs. When the animals were challenged, there would be fewer OVA-specific iTregs accumulated in the lung, resulting in more severe airway inflammation. Where TSLP would act to inhibit iTreg generation during natural allergen exposure is still unclear. TSLP is an epithelia-derived cytokine and is upregulated in skin and lung of atopic dermatitis and asthma patients (3–5). The ability of TSLP exposure within the first 24 h to profoundly inhibit subsequent Treg development suggests it may function at sites of priming. TSLP might function in lymph nodes, where Tslp mRNA has been detected (44). TSLP may also function systemically, following local production and release into the circulation. Epidermal keratinocytes in mice with severe AD-like skin inflammation expressed high TSLP, leading to increased serum concentration of the cytokine (41, 42). More importantly, recent data showed that serum TSLP levels in children with atopic dermatitis were significantly higher than normal controls (22). The increased systemic TSLP, even at a low level that is unable to promote Th2 differentiation, could inhibit the induction of Ag-specific iTreg, altering mucosal tolerance against harmless Ags, and rendering these individuals more susceptible to the development of Th2-dominated immune responses upon subsequent allergen exposure.

**Disclosures**

The authors have no financial conflicts of interest.

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