Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells

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Compelling evidence suggests that the epithelial cell–derived cytokine thymic stromal lymphopoietin (TSLP) may initiate asthma or atopic dermatitis through a dendritic cell–mediated T helper (Th)2 response. Here, we describe how TSLP might initiate and aggravate allergic inflammation in the absence of T lymphocytes and immunoglobulin E antibodies via the innate immune system. We show that TSLP, synergistically with interleukin 1 and tumor necrosis factor, stimulates the production of high levels of Th2 cytokines by human mast cells (MCs). We next report that TSLP is released by primary epithelial cells in response to certain microbial products, physical injury, or inflammatory cytokines. Direct epithelial cell–mediated, TSLP–dependent activation of MCs may play a central role in “intrinsic” forms of atopic diseases and explain the aggravating role of infection and scratching in these diseases.

Atopic diseases, including asthma, atopic dermatitis (AD), and allergic rhinitis, are associated with a genetic predisposition to develop pro-inflammatory immune responses to harmless components of the environment. These aberrant immune responses are characterized by the development of CD4+ T lymphocytes producing Th2 cytokines (IL-4, IL-5, and IL-13) and inducing the production of IgE antibodies. The important roles of Th2 lymphocytes and IgE-dependent activation of tissue mast cells (MCs) in acute and chronic inflammation characterizing atopic diseases have been well established in clinical and animal models. The allergic inflammation involves the accumulation of a cellular infiltrate in the airway mucosa or the skin consisting of eosinophils, CD4+ T cells, MCs, DCs, and basophils (1, 2).

Compelling evidence was recently provided that thymic stromal lymphopoietin (TSLP), an epithelial cell–derived cytokine, may have a determinant role in the initiation and maintenance of the allergic immune response (3, 4). TSLP was initially shown to activate and instruct human CD11c+ DCs to promote the differentiation of naive CD4+ T cells into Th2 proinflammatory effectors, defined by the production of high levels of pro-allergic cytokines IL-4, IL-5, IL-13, TNF, and low levels of IL-10 (5, 6). The role of TSLP in allergic diseases was subsequently supported by the findings that it was specifically overexpressed in the acute and chronic lesions of AD patients and in the bronchi of asthmatic patients, where its level of expression correlated with the severity of the disease (3, 7). The ability of TSLP to act as the initiating cytokine at the top of a chain of immunological events that lead to the atopic syndrome was formally demonstrated in animal models (8–10). Overexpression of the TSLP gene specifically in airway epithelial cells or keratinocytes led to asthma- and AD-like diseases, respectively (9, 10). Moreover, increased expression of TSLP in the keratinocytes of mice genetically deficient in retinoic acid receptor or treated by topical application...
of vitamin D3 was correlated with the occurrence of typical immunological and histological features of AD (11, 12). However, the findings that induction of experimental dermatitis or asthma can occur in TSLP-transgenic mice lacking T cells (TCRβ−/− or RAG−/−) demonstrated that bronchial or cutaneous allergic diseases can occur in T cell– and IgE-deficient animals (9–11). These findings suggested to us that TSLP may directly activate effector cells of the innate immune system like MCs, which are known to play an important role in the pathogenesis of atopic diseases (13, 14). Here, we report that TSLP released by primary epithelial cells in response to clinically relevant stimuli directly activates human MCs inducing the production of high levels of Th2 proinflammatory cytokines.

RESULTS AND DISCUSSION

Human MCs express functional receptor for TSLP

The expression of each chain of TSLP receptor complex, i.e., the TSLP-binding chain (TSLP-R) and the IL-7Rα chain (15), was first examined on progenitor-derived MCs at the mRNA and protein levels. TSLP-R mRNA was expressed on MCs but not on T cells used as a control. IL-7Rα was expressed at lower levels on MCs than on T cells. Expression of TSLP receptor complex was indicated by double labeling with mAb to c-kit in tandem with mAbs to either TSLP-R or IL-7Rα (Fig. 1 A). Importantly, TSLP receptor was also expressed in vivo on MCs infiltrating the bronchial mucosa of asthmatic patients as revealed by immunostaining of biopsy specimen (Fig. 1 B). Initial observations revealed that only IL-1 but not TNF, IL-4, or IL-6 exerted a permissive effect on the activation of MCs by TSLP as illustrated by the production of IL-5 (Fig. 1 C). Moreover, the response to TSLP plus IL-1 was further enhanced by TNF but not by IL-4 or IL-6. All the in vitro–generated MC lines examined in this study (n = 19) responded to TSLP in the presence of IL-1/TNF, regardless of whether they were derived from the blood of atopic or nonatopic adults or umbilical cord blood. The response of MCs to TSLP was dose dependent (Fig. 1 D); it was already detectable after 6 h of culture and reached plateau at 24 h (Fig. 1 E). This response was TSLP specific and mediated by TSLP-R. Indeed, (a) it was specifically suppressed by neutralizing mAb to TSLP or TSLP-R (Fig. 1, F and G), and (b) the inhibitory activities of these mAbs were TSLP specific in that they had no effect on the production of CCL2, which was highly induced by stimulation with IL-1/TNF in the absence of TSLP (Fig. S1 A, available at http://www.jem.org/cgi/content/full/jem.20062211/DC1).

TSLP stimulation of MCs induces cytokine production but not mediator release

Typically, IgE-dependent MC activation results in the liberation of granule-associated mediators such as histamine and tryptase, the synthesis of lipid mediators such as PGD2 and LTC4, and the synthesis of a wide spectrum of cytokines and chemokines. MC activation is not a “yes or no” phenomenon, and similar to several other MC activators (for review see reference 16), TSLP did not induce MC degranulation or the release of lipid mediators (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20062211/DC1), even when used at various concentrations with or without IL-1/TNF. In contrast, very high levels of the proinflammatory cytokines/chemokines IL-5, IL-13, IL-6, GM-CSF, CXCL8, and CCL1 were released after 24 h of MC stimulation by TSLP in the presence of IL-1/TNF (Fig. 2). Regardless of the experimental conditions, the following cytokines/chemokines,
including IL-4, IL-9, IL-12, IL-15, IFN-γ, CXCL10, CCL24, CCL17, CCL13, CCL22, and CCL5, were either undetectable or present at very low levels (<70 pg/ml). Stimulation of MCs with IL-1/TNF induced the release of high levels of CCL2 and CCL3, and this was not affected by TSLP (CCL2: 3,512 ± 346 pg/ml vs. 3,912 ± 669 pg/ml with TSLP; CCL3: 3,921 ± 725 pg/ml vs. 3,415 ± 483 pg/ml with TSLP). Collectively, these data indicated that in inflammatory conditions mimicked by the presence of IL-1 and TNF, TSLP is a potent activator of MCs leading to the production of very high levels of proinflammatory Th2 cytokines and chemokines that are reportedly sufficient to induce and maintain an allergic phenotype. For instance, the perfusion of IL-13 induces an asthma-like phenotype characterized by eosinophilic inflammation, bronchial hyperreactivity, and airway remodeling (17). Given the important role of TNF in severe asthma (18), it is of note that this cytokine was released at high levels by MCs stimulated with IL-1 and TSLP (not depicted). The proinflammatory activity of TSLP was further indicated by its suppressive activity on the production of TGF-β. This finding together with the observation that TGF-β inhibits the response to TSLP (Fig. S3) suggests a negative regulatory feedback between these two cytokines. In contrast to TGF-β, the production of IL-10 was enhanced by TSLP and exogenous IL-10 did not affect the MC response to TSLP (not depicted). IL-10 is overexpressed in the lesional skin of AD patients (19) where it inhibits the production of antimicrobial peptides, thereby contributing to the microbial colonization of the skin (20).

Human epithelial cells produce biologically active TSLP

Given that overexpression of TSLP in the airway epithelial cells induces experimental asthma (9), and that TSLP mRNA is overexpressed in the bronchial mucosa of asthmatic patients (7), we attempted to identify stimuli capable of inducing TSLP production by human airway epithelial cells. To this end, primary small airway epithelial cells (SAECs) were stimulated with: (a) a cocktail of IL-1 and TNF to mimic the inflammatory microenvironment, and (b) bacterial peptidoglycan (PGN) and TLR ligands such as lipoteichoic acid (LTA) from *Bacillus subtilis*, poly I:C (mimicking viral double-stranded RNA), LPS, imiquimod, and CpG. TSLP was produced only in response to the inflammatory cytokines, PGN, LTA, and poly I:C (Fig. 3 A and Fig. S4, which is available at http://www.jem.org/cgi/content/full/jem.20062211/DC1). The failure of SAEC to respond to LPS, imiquimod, and CpG was explained by the lack of expression of the corresponding TLR’s mRNA (Fig. 3 B). The supernatant fluids of activated SAECs promoted the TSLP-dependent proliferation of a BAF cell line transfected with the human TSLP receptor complex (Fig. 3 C). Moreover, the low levels of TSLP present in these culture supernatants were sufficient when used together with IL-1/TNF to induce IL-13 and IL-5 production from MCs (Fig. 3, D–F, and not depicted). Native SAEC-derived TSLP was active at much lower concentrations (50–100 pg/ml; see Fig. 3 A) than recombinant TSLP (10 ng/ml) used as a positive control (Fig. 3 D). This finding suggested that activated SAECs may produce additional factors that act to co-stimulate the response of MCs to TSLP; alternatively, it could reflect a difference in the

![Figure 2. TSLP-stimulated secretion of cytokines and chemokines by MCs.](image-url)
intrinsinc activity of native and recombinant TSLP. It is of note that SAECs stimulated with cytokines, PGN, or polyI:C did not produce detectable IL-5 or IL-13. Moreover, PGN or polyI:C induced the production of very low (≤70 pg/ml) or undetectable levels of IL-5 or IL-13 by MCs, even when used together with IL-1α/TNFα. The observation that bacterial and viral products induce TSLP production by SAECs may be related to the well-documented aggravating role of infection in allergic as well as intrinsic bronchial asthma. For example, 60–80% of asthma exacerbations in children and adults are caused by rhinovirus infection (21). Rhinoviruses, like several other single-stranded RNA viruses, synthesize double-stranded RNA during their replication, thereby engaging TLR3 and initiating signaling cascades leading to cytokine production (22). The up-regulation of TSLP by bacterial products is, however, not restricted to airway epithelial cells as it has been shown in intestinal epithelial cells (23). TSLP activation of MCs may also contribute to the aggravation of AD resulting from skin colonization by Staphylococcus aureus (1). Thus, certain bacterial, viral, and nonspecific inflammatory stimuli (IL-1α/TNFα) may activate airway epithelial cells to produce TSLP in sufficient amounts to stimulate MCs and thereby initiate and/or aggravate allergic inflammation.

MC activation by skin–derived TSLP

Because TSLP protein is reportedly overexpressed at the lesional sites of AD (3), we examined the possible involvement of TSLP-induced MC activation in this disease. To this end, biopsy fragments of lesional and nonlesional skin from AD patients were examined for their ability to directly stimulate MCs in co-culture experiments. As seen in Fig. 4 A, lesional skin induced IL-13 production by MCs in a TSLP-dependent manner, whereas nonlesional skin from the same patients was less active. Moreover, TSLP mRNA levels were higher in biopsy fragments from lesional than nonlesional skin (Fig. 4 B). The finding that nonlesional skin was active on MCs led us to test whether TSLP production was a feature of atopy or was induced by the physical trauma of the skin resulting from the biopsy. The latter possibility was supported by the finding that skin fragments of normal individuals released TSLP protein after 24 h of culture in sufficient quantities to stimulate MCs (Fig. 4 C). No such activity was elicited by supernatant fluids collected after 1 h of skin culture. Because normal skin reportedly does not express detectable TSLP protein (3), the data suggest that TSLP was induced during the culture of skin explants. This view was supported by the finding of increasing TSLP mRNA and protein expression over time in the skin cultures (Fig. 4, D and E). A similar result was obtained in experiments examining TSLP mRNA and protein expression over time in mouse skin punch biopsies (not depicted). The production of TSLP together with several proinflammatory cytokines after physical trauma may account for the aggravating role of scratching in atopic eczema (1).

An emerging hypothesis regarding asthma and AD is that they are epithelial cell diseases initiated by the epithelial cells themselves via the production of TSLP (3). In the present study we have identified several clinically relevant stimuli leading to TSLP production by primary human airway epithelial cells. We have further shown that these stimulated epithelial cells release TSLP in sufficient amounts to activate, in synergy with IL-1α/TNFα, MCs to produce high levels of Th2 cytokines. These findings provide a possible mechanism to account for the induction of atopic-like diseases in T cell– and IgE-deficient mice expressing a TSLP transgene or submitted to topical application of vitamin D3 on the skin (9, 11). Direct epithelial cell–mediated and TSLP-dependent activation of MCs may be implicated in the initiation and perpetuation of so-called “intrinsinc” asthma or eczema in ~20–30% of patients (24). Such patients have normal serum IgE concentration and negative skin prick test
PGD2 and LTC4, as well as release with cytokines or PMA/ionomycin as a kine release. MCs were incubated for 30 min for histamine and 90 min for β-hexosaminidase, cytokine, and chemokine release. Assessment of mediator, TSLP (Amgen).

cytokines included: IL-1β, TNF (R&D Systems), and recombinant (M385; Amgen); anti–IL-10 (American Type Culture Collection). Recombinant cytokines/neutralizing antibodies as indicated. The antibodies used include:

MC cultures. All studies were approved by the ethics committee of CHUM Research Center. Human peripheral blood- or cord blood-derived CD34+ progenitor cells were isolated and cultured as described previously (26). After 10–12 wk of culture, >98% of cells were stained for c-kit (Becton Dickinson), FcεRI (eBioscience), and tryptase (Chemicon). 2 x 10^5/0.2 ml MCs were cultured in 96-well flat-bottom plates for 24 h in the presence of exogenous cytokines/neutralizing antibodies as indicated. The antibodies used include: anti–IL-7Rα (R&D Systems); anti–TSLP-R (M505; Amgen); anti-TSLP (M385; Amgen); anti–IL-1α (American Type Culture Collection). Recombinant cytokines included: IL-1β, TNF (R&D Systems), and recombinant TSLP (Amgen).

Assessment of mediator, β-hexosaminidase, cytokine, and chemokine release. MCs were incubated for 30 min for histamine and 90 min for PGD2 and LTC4, as well as release with cytokines or PMA/ionomycin as a positive control, and ELISA was performed (ImmunoTech and Cayman Chemical) according to the manufacturers’ instructions. β-Hexosaminidase release was analyzed as described previously (27). IL-4, IL-5, IL-6, CXCL8, IL-9, IL-10, IL-12, IL-13, IL-15, CCL24, CCL1, IFN-γ, CXCL10, GM-CSF, CCL22, CCL3, CCL2, CCL13, CCL5, CCL17, and TGF-β were examined in supernatants harvested after 24 h of MC activation via commercial kits. All assays were conducted in triplicates.

Real-time quantitative PCR. Primary SAECs (Clonetics) were grown to confluence and stimulated in the presence of 25 ng/ml TNF/10 ng/ml IL-1α, 100 μg/ml PGN from S. aureus, LTA from 2 μg/ml B. subtilis, 50 μg/ml polyI:C, 1 μg/ml LPS, 10 μg/ml imiquimod, or 5 μM CpG.

Proliferation assay. BAF cells stably expressing the human TSLP-R and IL-7Rα chains were cultured with SAEC supernatants in the presence or absence of neutralizing anti-TSLP antibody for 3 d, and proliferation was assessed by CyQUANT Cell Proliferation Assay kit (Invitrogen) according to the manufacturer’s instructions.

MCs and skin explant co-cultures. MCs were directly co-cultured with lesional and nonlesional skin fragments from AD patients for 24 h in the presence or absence of exogenous cytokines/neutralizing antibodies as indicated. Supernatants of skin explants from normal individuals undergoing plastic surgery were added to MCs in the presence or absence of exogenous cytokines/neutralizing antibodies as indicated.

Statistical analysis. Student’s paired t test and ANOVA (Tukey-Kramer Multiple Comparisons test) were used to determine the statistical significance of the data.

Online supplemental material. Fig. S1 shows the lack of effect of anti-TSLP mAb on MC response to cytokines and on expression of TSLP mRNA.

Figure 4. MC activation by skin-derived TSLP. (A) MCs were cultured with or without lesional or nonlesional skin fragments from AD patients in the presence of IL-1β/TNF with or without neutralizing mAb to TSLP. IL-13 and IL-5 (not depicted) were measured in the supernatants after 24 h of culture. (B) TSLP mRNA was assessed in the lesional and nonlesional skin of AD patients by real-time PCR. (C) Skin explants from nonallergic patients undergoing plastic surgery were minced and cultured for 24 h. Their cell-
Fig. S2 illustrates the failure of TSLP to stimulate MC degranulation and eicosanoid production. Fig. S3 illustrates the suppressive effect of TGF-β on TSLP response, and Fig. S4 shows the induction of TSLP production by SAECs in response to specific TLR2 ligand. The online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20062211/DC1.

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