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TSLP in Epithelial Cell and Dendritic Cell Cross Talk

Yong-Jun Liu
Department of Immunology, Center for Cancer Immunology Research, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas

Abstract

Dendritic cells (DCs) are professional antigen-presenting cells that have the ability to sense infection and tissue stress, sample and present antigen to T lymphocytes, and instruct the initiation of different forms of immunity and tolerance. The functional versatility of DCs depends on their remarkable ability to translate collectively the information from the invading microbes, as well as their resident tissue microenvironments. Recent progress in understanding Toll-like receptor (TLR) biology has illuminated the mechanisms by which DCs link innate and adaptive antimicrobial immune responses. However, how tissue microenvironments shape the function of DCs has remained elusive. Recent studies of TSLP (thymic stromal lymphopoietin), an epithelial cell-derived cytokine that strongly activates DCs, provide strong evidence at a molecular level that epithelial cells/tissue microenvironments directly communicate with DCs, the professional antigen-presenting cells of the immune system. We review recent progress on how TSLP expressed within thymus and peripheral lymphoid and nonlymphoid tissues regulates DC-mediated central tolerance, peripheral T cell homeostasis, and inflammatory Th2 responses.

1. INTRODUCTION

Epithelium is a tissue composed of layers of epithelial cells that line the cavities and surfaces of structures throughout the body, including the skin, lungs, the gastrointestinal tract, the reproductive and urinary tracts, and the exocrine and endocrine glands. Functions of epithelial cells include secretion, absorption, protection, transcellular transport, sensation detection, and selective permeability. Most immunologists rarely think about epithelial cells, with the exception of thymologists (immunologists working on thymus gland). The role of epithelial cells in the cortical region and medulla region of the thymic gland in T cell development have been one of the central focus of immunology for the past several decades (Anderson *et al.*, 2007). Epithelial cells in the skin, gut, and lung have long been suspected to play a key role in shaping the local and systemic immune responses (Holgate, 2007; Kato and Schleimer, 2007; Stingl, 1991; Xu *et al.*, 2007). However, how epithelial cells regulate immune homeostasis at the steady state and during immune response to infection and in disease states in the periphery have been relatively unclear. In this article, we will review the current progress on the biology of TSLP in the communication between epithelial cells and dendritic cells (DCs) in the development of the immune system, the maintenance of the immune homeostasis and the regulation of the immune responses.

2. THYMIC STROMAL LYMPHOPOIETIN (TSLP) AND TSLP RECEPTOR (TSLPR)

TSLP was first identified as an activity in conditioned medium super-natants from the mouse thymic stromal cell line, Z210R.1 that supported the long-term growth of a pre-B cell line and enhanced the proliferation of unfractionated thymocytes to suboptimal concentrations of anti-CD3 anti-bodies *in vitro* (Friend *et al.*, 1994). Subsequent expression cloning revealed that the mouse TSLP (mTSLP) is a member of the hematopoietic cytokine

family (Sims *et al.*, 2000). A cDNA clone encoding human TSLP (hTSLP) was isolated using database search methods (Quentmeier *et al.*, 2001; Reche *et al.*, 2001). Sequence prediction revealed a similar four-helix structured cytokine with two *N*-glycosylation sites and six cysteine residues. hTSLP and mTSLP exhibit poor homology with only 43% amino acid identity. TSLP is expressed mainly in the lung, skin, and gut (Reche *et al.*, 2001). TSLP receptor (TSLPR) is a heterodimeric receptor complex that consists of TSLPR and the IL-7α. The TSLPR chain is a member of the hematopoietin receptor family and binds to TSLP at low affinity. A combination of TSLPR and IL-7α chain results not only in high-affinity binding but also in SATT3 and STAT5 activation (Pandey *et al.*, 2000; Park *et al.*, 2000; Reche *et al.*, 2001; Fig. 1.1). hTSLP and mTSLPR share only 39% amino acid identity.

3. TSLP IN LYMPHOCYTE DEVELOPMENT IN MICE

Although mTSLP was identified and cloned based on a biological activity from a thymic epithelial cell line in supporting the growth of early B and T progenitors (Friend *et al.*, 1994; Levin *et al.*, 1999; Ray *et al.*, 1996; Sims *et al.*, 2000), $Tslpr^{-/-}$ mice display apparently normal T and B cell development (Al-Shami *et al.*, 2004). Therefore, mTSLP has been regarded as “an uninteresting weak brother of IL-7.” However, subsequent studies demonstrated that the target cells of TSLP and IL-7 are different. In bone marrow, IL-7 acts mainly on early lymphoid progenitors and pre-pro-B progenitors, TSLP acts on relatively late stage B cell progenitors, specifically at large pre-B stage that already expresses the pre-B cell receptor. Interestingly, fetal liver pro-B cells but not bone marrow-derived pro-B cells respond to TSLP (Vosshenrich *et al.*, 2003, 2004). TSLP displays an activity in supporting the growth of mouse $CD4^-$ $CD8^-$thymocytes in the presence of IL-1β in culture (Sims *et al.*, 2000). Administration of TSLP into γ_c-deficient mice led to a 5–10-fold increase in the thymus cell numbers with an early increase in the DP cells at one week, followed by an increase in the $CD4^+$ SP cells (Al-Shami *et al.*, 2004). More recent studies suggest that TSLP directly stimulate the thymic $CD4^+$ $CD8^-$ $CD25^-$ thymocytes to differentiate into Foxp3+ Treg cells (Lee *et al.*, 2008; Mazzucchelli *et al.*, 2008).

4. TSLP ACTIVATES HUMAN MYELOID DENDRITIC CELLS

Following the identification of hTSLP and TSLPR, hTSLP was found to dramatically and uniquely activate human $CD11c^+$ myeloid DCs (mDC). The initial observation that human monocytes express TSLPR and respond to hTSLP by producing chemokines TARC was found to be contributed by the contaminating mDCs in the monocyte preparations (Reche *et al.*, 2001; Soumelis *et al.*, 2002). The ability of mDCs to respond to TSLP is consistent with the finding that mDC express the highest levels of TSLPR at the both mRNA (Soumelis *et al.*, 2002) and protein levels among all human hematopoietic cell types (Liu Y.-J., unpublished observations). Because DCs represent the professional antigen-presenting cells, the central research focus on TSLP was then shifted from its role in regulating early lymphocyte development in the central lymphoid organs to its function in regulating DC-mediated immune responses in the peripheral lymphoid organs.

5. TSLP IN ALLERGIC INFLAMMATION

5.1. TSLP induces innate allergic immune responses by targeting mDCs, mast cells, and NK T cells

Like all stimuli that activate mDCs, including CD40L and Toll-like receptor (TLR) ligands, such as bacterial LPS, poly I:C, and R848, TSLP strongly upregulates the expression of MHC class II, CD54, CD80, CD83, CD86, and DC-lamp on human mDCs. However, unlike CD40L and TLR ligands, TSLP does not stimulate mDCs to produce the Th1-polarizing

cytokine IL-12 and type 1 interferones or the proinflammatory cytokines TNF, IL-1β, and IL-6 (Table 1.1; Soumelis *et al.*, 2002). Interestingly, TSLP treatment causes mDCs to produce large amounts of the chemokines IL-8 and eotaxin-2, which attract neutrophils and eosinophils, followed by production of TARC (CCL17) and MDC (CCL22), which attract Th2 cells (Table 1.1). A more recent study showed that hTSLP potently activates human mast cells to produce IL-5, IL-6, IL-13 and GM-CSF, and IL-8 and I-309, in the presence of IL-1β and TNF (Allakhverdi *et al.*, 2007). Another study showed that TSLP may potentially activate NKT cells to produce IL-13 in a mouse asthma model (Nagata *et al.*, 2007). These studies suggest that TSLP produced by epithelial cells may rapidly induce an innate phase of allergic inflammatory response by activating mDCs, mast cells, and NK cells to produce TH2 cytokines, chemokines, and proinflamatory cytokines. The role of TSLP in the triggering of an early innate phase of allergic inflammation is supported by an *in vivo* observation that TSLP can induce moderate airway inflammation in B and T cell deficient $RAG^{-/-}$ mice (Zhou *et al.*, 2005). TSLP induced innate phase of allergic immune response has three important consequences: (1) it induces a transient inflammatory response via IL-6, IL-13, and GM-CSG; (2) it recruits eosinophils via IL-5 and eotaxin-2, as well as neutrophils via IL-8; (3) it prepares for the local adaptive Th2 responses by producing TARC and MDCs which will attract Th2 cells generated subsequently by TSLP-activated mDCs (TSLP-DC) during the adaptive phase of allergic immune responses; and (4) it educates a unique population of mDCs that acquire the ability to induce naïve $CD4^+$ T cells to differentiate into inflammatory Th2 cells.

5.2. TSLP triggers adaptive allergic immune responses via mDCs

5.2.1. TSLP-DC induce inflammatory Th2—When TSLP-DCs are used to stimulate naive allogeneic $CD4^+$ T cells *in vitro*, they induce a unique type of Th2 cell that produces the classical Th2 cytokines IL-4, IL-5, and IL-13 and large amounts of TNF, but little or no IL-10 (Soumelis *et al.*, 2002). Although not typically considered a Th2 cytokine, TNF is prominent in asthmatic airways, and genotypes that correlate with increased TNF secretion are associated with an increased risk of asthma (Moffatt and Cookson, 1997), suggesting that TNF plays an important role in the development of asthma and allergic inflammation. In addition to inducing the production of Th2 cytokines and TNF, $CD4^+$ T cells activated by TSLP-DCs produce decreased levels of IL-10 and IFN-γ, two cytokines known to downregulate Th2 inflammation (O'Garra, 1998). IL-10, although initially classified as a Th2 cytokine, counteracts inflammation, and is produced at decreased levels in bronchoalveolar lavage fluid from atopic patients compared with normal subjects (Borish *et al.*, 1996). In addition, recent studies show that DC- or T cell–derived IL-10 prevents airway hypersensitivity after allergen exposure (Akbari *et al.*, 2001; Oh *et al.*, 2002). Because of their unique profile of cytokine production, we propose that Th2 cells induced by TSLP-activated DCs be called inflammatory Th2 cells, in contrast to the conventional Th2 cells (Fig. 1.2). The pathogenic T cells involved in allergic diseases such as atopic dermatitis and asthma are likely to be inflammatory Th2 cells. Conventional Th2 cells that produce IL-4, IL-5, IL-13, and IL-10, but little TNF, may not be involved in promoting allergic diseases but are induced in many circumstances, including when APCs or T cells are treated with immunosuppressive drugs and when T cells are triggered by low-affinity TCR ligands (Boonstra *et al.*, 2001; Constant and Bottomly, 1997; de Jong *et al.*, 1999).

5.2.2. TSLP-DC express a Th2 polarizing molecule OX40L—In an attempt to identify the molecular mechanism by which TSLP-DCs induce naive $CD4^+$ T cells to differentiate into TNF-producing inflammatory Th2 cells, our group performed gene expression analysis on immature human mDCs that were either resting or were activated by TSLP, poly I:C, or CD40L. This analysis showed that only TSLP induces human mDCs to express the TNF superfamily protein OX40L at both the mRNA and protein levels (Ito *et al.*,

2005). The expression of OX40L by TSLP-DCs was important for the induction of inflammatory Th2 cells, as blocking OX40L with a neutralizing antibody inhibited the production of Th2 cytokines and TNF and enhanced the production of IL-10 by the $CD4^+$ T cells. Consistent with these results, we found that treating naive T cells with recombinant OX40L promoted the production of TNF but inhibited the production of IL-10. In other words, signals triggered by OX40L induced the generation of inflammatory Th2 cells. A recent study demonstrates that OX40 signaling directly induces Th2 lineage commitment by inducing NFATc1, which triggers IL-4 production and then IL-4-dependent GATA-3 transcription (So *et al.*, 2006). In addition, blocking OX40L was shown to inhibit TSLP-induced asthma in a mouse model *in vivo* (Seshasayee *et al.*, 2007).

5.2.3. TSLP-DC provide a permissive condition for Th2 development—One of the key features of TSLP-DC is their expression of all the major costimulatory molecules and OX40L that is uncoupled with IL-12 production. In the presence of exogenous IL-12, TSLP-DC or recombinant OX40L loses the ability to induce Th2 differentiation. We thus conclude that TSLP-activated DCs create a Th2-permissive microenvironment by upregulating OX40L without inducing the production of Th1-polarizing cytokines. The dominance of IL-12 over OX40L may provide a molecular explanation for the hygiene theory, which proposes that microbial infections that trigger Th1 responses may decrease the subsequent development of Th2-driven atopy. Historically, two models have been proposed to explain how Th2 development is initiated: (1) Th2 differentiation requires a positive Th2-polarizing signal, or (2) Th2 development is initiated by a default mechanism in the absence of IL-12 (Eisenbarth *et al.*, 2003; Kapsenberg, 2003; Moser and Murphy, 2000; Sher *et al.*, 2003; Fig. 1.3). Our findings suggest that the two previously proposed models are not mutually exclusive and that Th2 differentiation requires a positive polarizing signal, such as OX40L as well as a default mechanism (the absence of IL-12).

5.3. TSLP association with human atopic dermatitis and asthma

Early studies showed that TSLP mRNA is highly expressed by human primary skin keratinocytes, bronchial epithelial cells, smooth muscle cells, and lung fibroblasts but not by most hematopoietic cells, including B cells, T cells, NK cells, granulocytes, macrophages, monocytes, or DCs (Soumelis *et al.*, 2002). Interestingly, mast cells activated by IgE receptor cross-linking expressed high levels of TSLP, suggesting an additional cell type that may help trigger allergic inflammation. TSLP protein, examined by immunohistology on cryopreserved tissue sections, is undetectable in normal skin or nonlesional skin in patients with atopic dermatitis but is highly expressed in acute and chronic atopic dermatitis lesions (Soumelis *et al.*, 2002). TSLP is expressed mainly in keratinocytes of the apical layers of the epidermis, suggesting that TSLP production is a feature of fully differentiated keratinocytes (Fig. 1.4). TSLP is not found in skin lesions from patients with nickel-induced contact dermatitis or disseminated lupus erythematosus (Soumelis *et al.*, 2002). Interestingly, TSLP expression in patients with atopic dermatitis is associated with Langerhans cell migration and activation *in situ* (Fig. 1.4), suggesting that TSLP may contribute directly to the activation of these cells, which could then migrate into the draining lymph nodes and prime allergen-specific Th2 responses (Soumelis *et al.*, 2002). A more recent study showed by *in situ* hybridization that TSLP expression is increased in asthmatic airways and correlates with both the expression of Th2-attracting chemokines and with disease severity (Ying *et al.*, 2005), providing the first link between TSLP and human asthma.

5.4. TSLP in allergic inflammation *in vivo*

In 2005, 3 years after the initial report on the function of TSLP in DC-induced Th2 responses in culture and association of TSLP *in situ* with alergic diseases in humans, three groups provided the genetic *in vivo* data showing that TSLP is critical for the development

of allergic inflammation in mouse models. While Ziegler's group demonstrated that tissue specific over expression of TSLP in lung and skin induces asthma and atopic dermatitis, respectively (Yoo *et al.*, 2005; Zhou *et al.*, 2005), Leonard's group showed that TSLPR knock out mice fail to develop airway inflammatory disease in an asthma model (Al-Shami *et al.*, 2005). Chambon's group reported that retinoid X receptor ablation in adult skin keratinocytes triggers TSLP production and atopic dermatitis in mice. Over expression of TSLP in skin keratinocytes induces atopic dermatitis (Li *et al.*, 2005). A recent study further showed that administration of TSLP protein directly into mouse airway causes asthmatic inflammation, which could be blocked by administration of neutralizing antibody to OX40L (Seshasayee *et al.*, 2007). This study also showed that in a rhesus monkey dust-mite-induced asthma model, there are elevated expressions of both TSLP and OX40L in the lung. Treatment with antihuman OX40L monoclonal antibody reduces the numbers of infiltrating cells and levels of Th2 cytokine IL-5 and IL-13 in the lung (Seshasayee *et al.*, 2007).

A more recent study suggests that TSLP may also play a key role in the development of a protective Th2-immunity in the gut, which is critical for controlling parasite infection, as well as maintaining mucosal immune homeostasis by limiting Th1 or Th17 immune responses (Zaph *et al.*, 2007). During parasite trichuris infection, intestinal epithelial cells deficient in IKK-β expression fail to produce TSLP, leading to impaired protective Th2 responses and uncontrolled Th1 and Th17 inflammatory responses (Zaph *et al.*, 2007).

5.5. Does TSLP directly activate CD4$^+$ T cells and induce Th2 differentiation?

Although it is more established in both human and mice that TSLP can activate CD4$^+$ T cells and induce Th2 differentiation via mDCs, whether TSLP can directly induce CD4$^+$ T cell proliferation and Th2 differentiation and the relative contribution of the direct effect versus indirect effect of TSLP on CD4$^+$ T cells are still unclear.

Several studies in mice suggest that TSLP may indeed have direct effects on CD4$^+$ T cells. The finding that CD4$^+$ T cells from TSLPR Ko mice expanded less efficiently than WT CD4$^+$ T cell in irradiated hosts suggest that TSLP does play a role directly in the CD4$^+$ T cell homeostasis (Al-Shami *et al.*, 2004). However, it is unclear whether TSLP plays a role in maintaining CD4$^+$ T cell survival or in promoting CD4$^+$ T cell proliferation, or both. *In vitro* culture of naïve CD4$^+$ T cells with anti-CD3 and anti-IFN-γ showed that addition of TSLP could prime cultured CD4$^+$ T cells to produce IL-4, IL-5, and IL-13 (Omori and Ziegler, 2007). However, TSLP does not induce Stat6 phosphrylation in the cultured CD4$^+$ T cells, suggesting that the ability of TSLP to induce IL-4 production by cutlured CD4$^+$ T cells was Stat6-independent. Paradoxically, TSLP fails to induce the generation of IL-4 producing cells from the Stat6$^{-/-}$ CD4$^+$ T cells, suggesting the TSLP-mediated Stat6-independent mechanisms is not enough for TH2 differentiation (Omori and Ziegler, 2007). In a murine model of Th2 immune responses induced by injection of a protease allergen papain in the footpads, basophils were found to be directly activated and recruited to the draining lymph nodes. Activated basophils produced both IL-4 and TSLP. *In vivo* neutralization of TSLP by monoclonal antibody leads to considerable inhibition of Th2 differentiation without affecting DC maturation and migration, thus suggesting that TSLP released by basophils play a critical role in direct Th2 differentiation. This study further shows that *in vitro* cutlure of naïve CD4$^+$ T cells with anti-CD3, TSLP was found to induce cultured T cells to produce Th2 cytokines and expression of GATA-3 (Sokol *et al.*, 2007).

A recent study in humans showed that human naïve CD4$^+$ T cells express low levels of TSLPR after 3 days of activation by anti-CD3 and anti-CD28. TSLP activates Stat5 and promotes the proliferation of activated CD4$^+$ T cells (Rochman *et al.*, 2007).

Although the above studies all suggest that TSLP can directly induce $CD4^+$ T cell proliferation or Th2 differentiation, the fact is that the levels of TSLPR expression on activated $CD4^+$ T cells is extremely low when compared with that expressed by mDCs in the human system. Because both human and mouse mDCs can express CD4, and TSLP activated mDCs could induce potent naïve $CD4^+$ T cell proliferation at even 1:150 DC/T cell ratio, the possible contribution of the few mDCs in the culture should be carefully examined in both human and mouse system.

5.6. Regulation of TSLP expression in allergic inflammation

Experimental evidence in both human and mice suggest that TSLP derived from epithelial cells represent an early trigger of allergic inflammation. Fibroblasts, smooth muscle cells, basophils, and mast cells have also been implicated in having the ability to proudce TSLP (Soumelis *et al.*, 2002). However, how TSLP expression in epithelial cells and other cells is triggered upon allergen exposure remains elusive. Using mouse genetic approach, Chambon's group demonstrated that retinoid X receptor/retinoid acid receptor complex and retinoid X receptor/vitamin D receptor complex negatively regulate the expression of TSLP in skin keratinocytes at the steady state. Deletion of retinoid X receptor (α/β) or blocking their transcription repression function by vitamin D3 or its analog led to uncontrolled expression of TSLP in skin kerotinocytes and atopic dermatitis (Li *et al.*, 2006; Yoo *et al.*, 2005). Ziegler's group found that TSLP promoter in humans and mice contain a NF-κB site. TNF and IL-1β were shown to stimulate human epithelial cell lines to produce TSLP in a NF-κB-dependent fashion (Lee and Ziegler, 2007). The importance of NF-κB activation in TSLP production was further demonstrated *in vivo* by a experiment showing that mice with a specific deletion of IKK-β in intestinal epithelial cells have reduced expression of TSLP at the steady state or upon parasite infection (Zaph *et al.*, 2007). Other studies further showed that in addition to TNF and IL-1β, Th2 cytokines IL-4 and IL-13 and TLR3-ligand Poly I:C could also stimulate human epithelial cells to produce TSLP (Bogiatzi *et al.*, 2007; Kato and Schleimer, 2007). A recent study demonstrates that basophils produce TSLP in the allergen papin induced Th2 immune responses model (Sokol *et al.*, 2007). Another study showed that TSLP production by nasal epithelial cells depends on mast cells in a mouse allergic rhinitis model (Miyata *et al.*, 2008).

The link between allergen exposure and induction of TSLP is still missing. The identification of the putative innate receptors that potentially sense allergen and the link between these receptors to retinoid X receptor signaling and NF-κB activation may help to reveal the missing link.

6. TSLP IN PERIPHERAL $CD4^+$ T CELL HOMEOSTASIS

Over two decades ago, Nussenzweig and colleagues (Nussenzweig *et al.*, 1980) observed that mouse splenic DCs could induce the proliferation of autologous T cells in culture in the absence of exogenous antigens, a phenomenon referred to as a syngeneic mixed lymphocyte reaction. Investigators concluded from this study that DCs may present self-pMHC complexes to autologous T cells. A more recent study illustrated that exposure to self-pMHC on the surface of autologous DCs induces phosphorylation of TCR3 and ZAP-70 in $CD4^+$ T cells (Kondo *et al.*, 2001). Using a mouse model in which only peripheral DCs expressed MHC class II, Brocker (1997) further demonstrated that the homeostatic survival and proliferation of naive $CD4^+$ T cells depended on their interaction with peripheral DCs. Proliferation of naive $CD8^+$ T cells adoptively transferred into lymphopenic hosts was enhanced by cotransfer of syngeneic DCs (Ge *et al.*, 2002). Similarly, syngeneic DCs, but not B cells or macrophages, induced homeostatic proliferation of naive $CD8^+$ T cells *in vitro* (Ge *et al.*, 2002). Collectively, these studies suggest that DCs play a critical role in the maintenance of T cell homeostasis under normal physiological conditions. However, we do

not know if the ability of DCs to induce homeostatic T cell proliferation can be regulated or, if so, how.

By immunohistology, we found that TSLP is expressed by crypt epithelial cells of human tonsils and TSLP expression is closely associated with DC-lamp$^+$-activated DCs under normal physiological conditions (Watanabe *et al.*, 2004; Fig. 1.5). Because TSLP-activated DCs have the capacity to induce very strong expansion of naive CD4$^+$ T cells, we hypothesized that hTSLP expressed by the epithelial cells of peripheral mucosa lymphoid tissues may play a critical role in DC-mediated homeostatic proliferation of naive and memory T cells. Indeed, we found that only TSLP-activated mDCs, but not resting or mDCs activated by IL-7, CD40L, lipopolysaccharide (LPS), or poly I:C, could induce a robust and sustained expansion of autologous naive CD4$^+$ T cells without any exogenous antigens, cytokines, or fetal bovine serum (Watanabe *et al.*, 2004). This unique ability of TSLP-activated DCs correlates with their strong capacity to form prolonged conjugate with the autologous naive CD4$^+$ T cells and thus provides sustained proliferation and survival signals (Watanabe *et al.*, 2004). The expansion of the autologous naive CD4$^+$ T cells induced by TSLP-activated mDCs displays features of homeostatic expansion mediated by self-pMHC complexes: (1) It is dependent on MHC class II and costimulatory molecules CD80/CD86, but not on IL-7 or IL-15; (2) it is a polyclonal expansion, as indicated by the TCRVβ repertoire analyses and CFSE-labeling experiments; and (3) the expanded cells display central memory T cell phenotype (CD45R0$^+$CCR7$^+$ CD27$^+$CD62L$^+$) and have the potential to further expand and differentiate into either Th1 or Th2 effector cells (Watanabe *et al.*, 2004). A recent study suggests that a low level of TSLP constitutively produced by the mucosal epithelium is critical to condition mucosal DCs to have a noninflammatory phenotype and maintain mucosal homeostasis (Rimoldi *et al.*, 2005). In support of this model, decreased TSLP production was found to associate with Crohn's disease (Rimoldi *et al.*, 2005). Experiments in TSLPR-deficient mice suggest a similar role for TSLP in the maintenance of peripheral CD4$^+$ T cell homeostasis *in vivo* (Al-Shami *et al.*, 2004).

Current data suggest that TSLP may promote CD4$^+$ T cell homeostasis through both direct effect on CD4$^+$ T cells and indirect effect via DCs.

7. TSLP IN THE DEVELOPMENT OF REGULATORY T CELLS IN THYMUS

TSLP was originally cloned from mouse thymic epithelial cells, however, neither the type of epithelial cell expressing TSLP nor their function in thymus is known. The first clue for the possible function of TSLP in human thymus came from the observation that hTSLP was found that TSLP is selectively expressed by epithelial cells of the Hassall's corpuscles (HCs) within the human thymic medulla (Watanabe *et al.*, 2005; Fig. 1.6). The major function of TSLP in human thymus appears to activate a subpopulation of DCs in the thymic medulla. Indeed, we found that TSLP strongly activates mDCs isolated from human thymus, and TSLP expression by HCs is associated with an activated mDC subpopulation in the thymic medulla (Watanabe *et al.*, 2005; Fig. 1.6). Because thymus is not a peripheral lymphoid organ that is normally exposed to microbial infection or immune responses, this raised a question regarding the functions of TSLP or TSLP-activated DCs in the thymus. Our hypothesis that TSLP-activated mDCs may play a critical role in the secondary positive selection of medium- to high-affinity self-reactive thymocytes to differentiate into Tregs (Watanabe *et al.*, 2005) is based on the following considerations:

1. CD28 signaling is critical for Treg development in thymus (Salomon *et al.*, 2000), and TSLP may represent the only physiological signal to activate thymic DCs to express CD80 and CD86, the ligands for CD28, in the medulla of human thymus (Watanabe *et al.*, 2005);

2. TSLP-activated DCs induce a robust homeostatic proliferation of naive $CD4^+$ T cells owing to their unique ability to form strong and prolonged conjugates with autologous $CD4^+$ T cells (Watanabe *et al.*, 2004);
3. Using the same mechanisms of inducing peripheral T cell homeostatic proliferation, TSLP-activated DCs may provide strong survival signals to the medium- to high-affinity self-reactive T cells and therefore, switch negative selection to a secondary positive selection.

This hypothesis is supported by our recent experiments showing that TSLP-activated DCs, but not DCs stimulated with IL-7, CD40-L, or poly I:C nor unstimulated DCs (Med-DC), induce a vigorous expansion of $CD4^+$ $CD8^-$ $CD25^-$ thymocytes, and about 50% of the expanded cells differentiate into $CD4^+$ $CD8^-$ $CD25^-$ $Foxp3^+$ Tregs (Watanabe *et al.*, 2005). The ability of TSLP-DCs to induce the differentiation of $CD4^+CD8^-$ $CD25^-$ thymocytes into Tregs depends on IL-2 and CD28 signaling (Watanabe *et al.*, 2005). By immunohistology, we found that $CD4^+CD25^+$ Tregs are exclusively localized within the thymic medulla in close association with DC-$LAMP^+$/$CD86^+$-activated DCs and HCs (Watanabe *et al.*, 2005). These data suggest that human $CD4^+CD25^+$ Tregs are generated in the thymic medulla, in close association with DCs that appear to be activated by TSLP produced by epithelial cells of the HCs (Watanabe *et al.*, 2005).

On the basis of these findings, we proposed a new model of central tolerance, as illustrated in detail in Fig. 1.7, that has the following features:

1. It explains how thymic DCs can mediate both negative and positive selection.
2. It suggests that the fate of a T cell within the thymus also follows the two-signal model: When the high-affinity self-reactive T cells receive strong TCR signaling without adequate costimulatory signals from either medullary epithelial cells or immature DCs, they die by negative selection. However, when the high-affinity self-reactive T cells receive strong TCR signaling and multiple costimulatory/survival signals from the TSLP-activated DCs, they will be converted into Tregs by a secondary positive selection.
3. It is consistent with the *in vivo* localization of Tregs within thymic medulla.
4. It explains the biological function of TSLP expressed by the epithelial cells of HCs, and why both activated and nonactivated myeloid DCs are present in the thymic medulla.
5. It overcomes the limited ability of thymic epithelial cells to express all the organ-specific antigens. DCs have the potential to cross-present thymic-derived antigens, as well as to sample all peripheral antigens and then migrate and present these antigens in the thymus.

Several studies in mice shows that mTSLP strongly promotes the differentiation and expansion of $Foxp3^+$ Tregs in thymus and periphery (Besin *et al.*, 2008; Jiang *et al.*, 2006; Lee *et al.*, 2008). However, TSLPR-deficient mice do not appear to have abnormal Treg development. The precise role of TSLP in Treg development still remains to be established.

8. SUMMARY AND FUTURE PERSPECTIVES

The function of TSLP in both mouse and human is pleiotropic. The major cell type that responds to TSLP is mDC. TSLP represents the only factor that activates mDCs without inducing them to produce Th1-polarizing cytokines. This sterile/aseptic way of activating mDCs, in contrast to the way of activating DCs by different TLR ligands and TNF family members, may explain the uniqueness of TSLP-DC function. Further investigation on how

TSLP versus TLR-Ligands signal mDCs will be critical to understand the molecular basis of the functional plasticity of mDCs in directing different types of T cell responses.

Under normal physiological conditions, TSLP appears to play a critical role in $CD4^+$ T cell homeostasis in the peripheral mucosa-associated lymphoid tissues (Rimoldi *et al.*, 2005; Watanabe *et al.*, 2004) and in the positive selection and/or expansion of Tregs in the thymus (Watanabe *et al.*, 2005). The signals that control the steady state level of TSLP production are unknown, but may involve RXRα and RXRβ (Li *et al.*, 2005).

In inflammatory conditions, such as atopic dermatitis and asthma, epithelial cells markedly increase TSLP expression in response to inflammation. Although the link between allergen and TSLP production is still missing, TSLP production in epithelial cells can be triggered by virus via TLR3 or by TH2 plus proinflammatory cytokines through NF-κB activation. The increased local TSLP will activate DCs mast cells and NK cells to initiate the innate phase of allergic immune responses (Fig. 1.8). The TSLP-activated DCs migrate to the draining lymph nodes, priming $CD4^+$ T cells via OX40L to differentiate into inflammatory TH2 effector and memory cells and therefore initiate the adaptive phase of allergic immune responses (Fig. 1.8). When considering the pathophysiology and therapeutic targets of allergic diseases, both innate and adaptive phases of allergic immune response should be considered.

TSLP instructs mDCs to induce inflammatory Th2 cells in two ways. First, TSLP induces DC maturation without driving the production of the Th1-polarizing cytokine IL-12, thus creating a Th2-permissive microenvironment. Second, TSLP induces the expression of OX40L on DCs, which directly triggers the differentiation of inflammatory Th2 cells. The signaling pathway that is triggered by TSLP and leads to this unique Th2 phenotype is unknown, but it appears to involve STAT5 activation, independent of the classical NF-κB and MyD88 signaling pathways.

OX40L signaling has several important features. It triggers Th2 polarization independent of IL-4, promotes TNF production, and inhibits IL-10 production by the developing Th2 cells, but only in the absence of IL-12. In the presence of IL-12, OX40L signaling instead promotes the development of Th1 cells that, like inflammatory Th2 cells, produce TNF but not IL-10. This finding may help explain why blocking OX40/OX40L interaction reduces the severity of Th1-mediated autoimmune diseases (Croft, 2003)—the reason some immunologists are reluctant to accept OX40L as a Th2-polarizing factor. We now believe that this inhibition of Th1-induced pathology is due to the increased production of the immunosuppressive cytokine IL-10 and the decreased production of the inflammation-promoting cytokine TNF-α, which results from blocking OX40–OX40L interactions. On the basis of these recent studies, we propose the subdivision of Th2 cells into inflammatory Th2 cells that produce high levels of TNF but little IL-10, and conventional Th2 cells that produce little TNF but high levels of IL-10. Inflammatory Th2 cells, but not conventional Th2 cells, may be involved in allergic inflammatory diseases.

Our initial finding that epithelial cell-derived TSLP triggers DC-mediated inflammatory Th2 responses in humans together with the exciting *in vivo* studies reported in early 2006 suggest that TSLP represents a master switch of allergic inflammation at the epithelial cell–DC interface. TSLP should, therefore, be considered as a target for immunological intervention in the treatment of allergic diseases.

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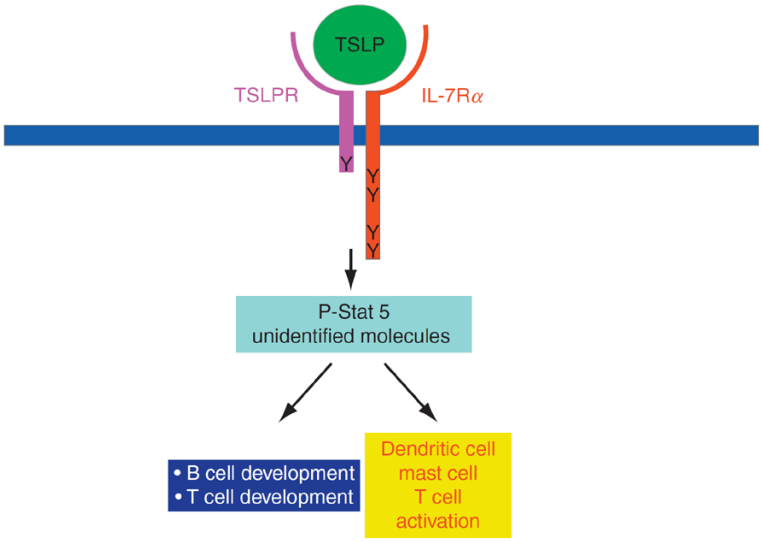

FIGURE 1.1.

TSLP and TSLPR structure and function. The TSLPR complex contains a heterodimer of TSLPR and IL-7Rα. TSLP stimulation induces activation and phosphorylation of STAT5 (P-STAT5), as well as activation of other as yet unidentified pathways. TSLP was discovered by its biological activity to promote B and T cell development. In the periphery, TSLP directly strongly activates DCs by upregulating MHC class I and II molecules and costimulatory molecules, promotes cell survival, and induces secretion of chemokines, which mediate different functions in central tolerance, T cell homeostasis and Th2 differentiation. In addition, TSLP may act directly on mast cells, NK cells, and $CD4^+$ T cells.

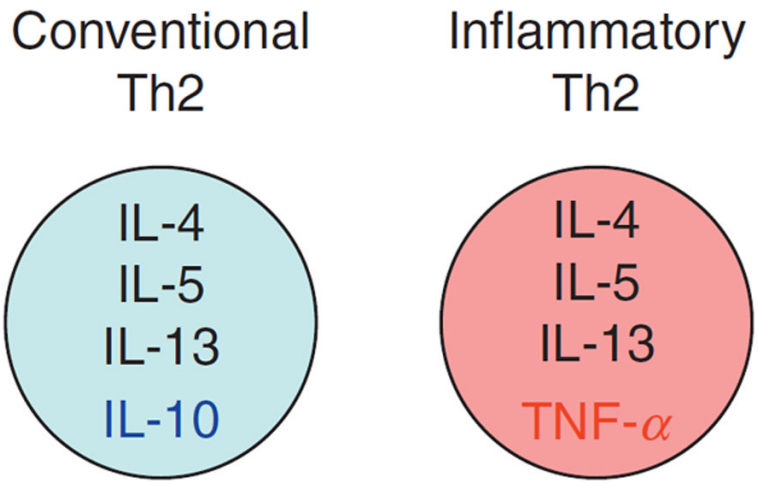

FIGURE 1.2.
Two types of Th2 cells defined by their IL-10 and TNF-α production. Conventional Th2 cells produce IL-4, IL-5, IL-13, and IL-10. Inflammatory Th2 cells produce IL-4, IL-5, IL-13, and TNF-α.

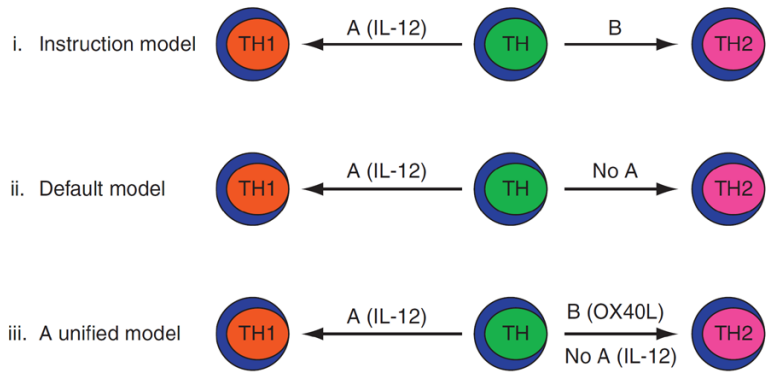

FIGURE 1.3.

Three models for the regulation of Th1 and Th2 differentiation. (A) Instruction model: Th1 differentiation requires a Th1-polarizing signal, and Th2 differentiation requires a Th2-polarizing signal. (B) Default model: Th1 differentiation requires a Th1-polarizing signal, and Th2 differentiation occurs spontaneously in the absence of the Th1-polarizing signal. (C) A unified model: Th1 differentiation requires a Th1-polarizing signal, and Th2 differentiation requires a Th2-polarizing signal. However, the Th1-polarizing signal is dominant over the Th2-polarizing signals. The Th2 signal can induce a Th2 response only in the absence of a Th1-polarizing signal.

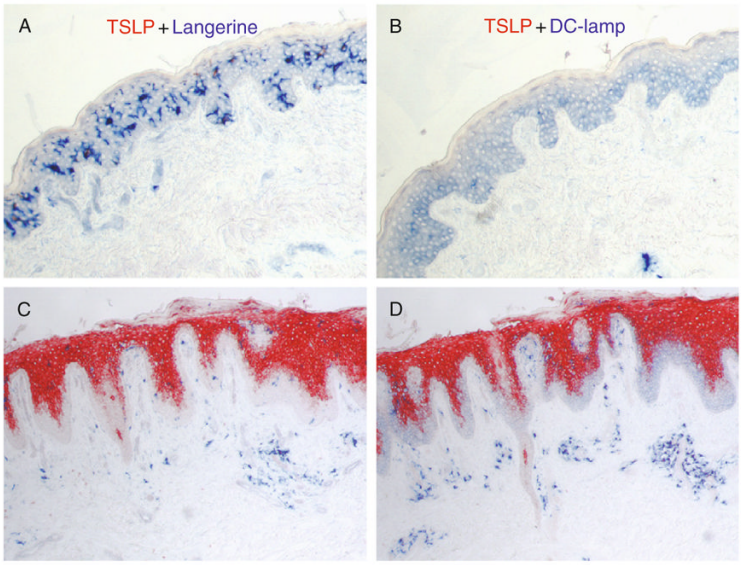

FIGURE 1.4.

TSLP expression in atopic dermatitis associates with Langerhans cell migration and activation. (A) Normal skin Langerin^{+} Langerhans cells in epidermis (blue staining) but does not express TSLP (thus no red staining). (B) Normal skin does not contain DC-lamp^{+}-activated DCs in epidermis and dermis nor does it express TSLP (thus no blue or red staining). (C) In skin lesion of atopic dermatitis, high expression of TSLP (red staining) is associated with the migration of Langerhans cells from epidermis to dermis. (D) The expression of TSLP (red staining) in skin lesion of atopic dermatitis is associated with the appearance of many DC-lamp^{+} activated DCs in dermis (blue staining).

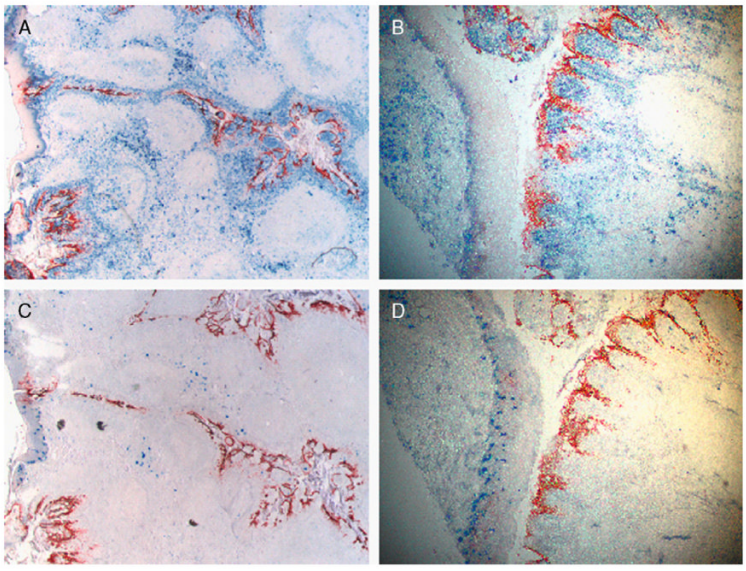

FIGURE 1.5.

Expression of TSLP in human tonsillar epithelial cells and its association with DC-lamp$^+$ activated DCs. (A, B) Double staining of TSLP (red staining) and DC-lamp (blue, an activated DC marker) shows expression of TSLP by crypt epithelial cells (red), which are in close association with DC-lamp$^+$ lymphocytes and DCs (blue; A, 100×; B, 200×). (C, D) Double staining of TSLP (red) and Langerin (blue, a Langerhans cell marker) shows TSLP expression (red) by crypt epithelial cells, but not by squamous epithelial cells characterized by the presence of Langerin-positive Langerhans cells (blue staining). Langerin-positive Langerhans cells within epidermis do not express DC-lamp (C, 100×; D, 200×).

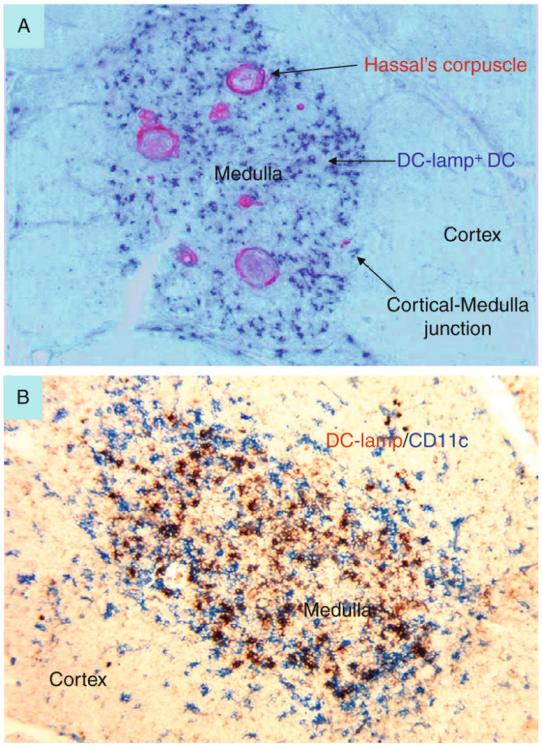

FIGURE 1.6.

TSLP expression in human thymus. (A) TSLP expression by HCs and thymic DC subpopulations. Epithelial cells of HCs that express TSLP (pink) are surrounded by the DC-lamp^{+}-activated DCs (dark blue) in the medulla of human thymus (100×). (B) Two subsets of DCs in human thymus. Human thymus contains a subset of CD11c^{+} DC-lamp^{-} immature DCs (blue) and a subset of CD11c^{+} DC-lamp^{+} activated DCs in the medulla of thymus (red brown; 100×).

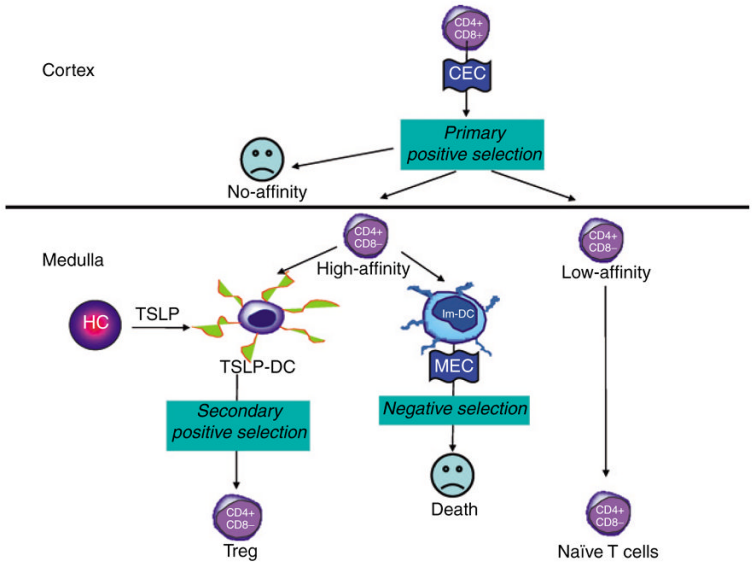

FIGURE 1.7.

A unified model of central tolerance in thymus. Developing T cells undergo the primary positive selection in the cortex by cortical epithelial cells. The positively selected T cells migrate into the medullary areas. The low-affinity self-reactive T cells may escape negative selection by medullary epithelial cells or immature DCs, and are exported to the periphery as naive conventional T cells. Majority of the high-affinity self-reactive T cells will undergo negative selection when binding antigen presented by medullary epithelial cells or immature thymic DCs. A small number of the high-affinity self-reactive T cells will undergo secondary positive selection when binding antigens presented by TSLP-activated thymic DCs.

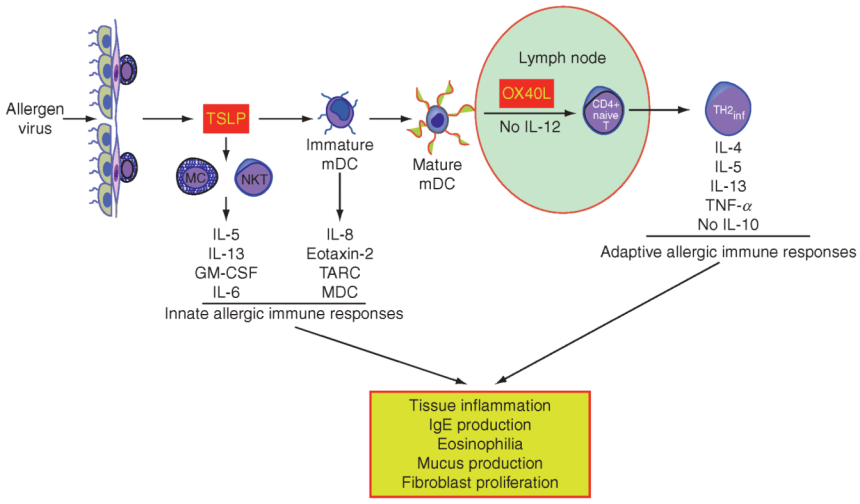

FIGURE 1.8.

TSLP initiates innate and adaptive phases of allergic inflammation. Insults from allergens or viruses trigger mucosal epithelial cells or skin cells (keratinocytes, fibroblasts, and mast cells) to produce TSLP. TSLP initiates the innate phase of allergic immune responses by activating immature DCs to produce the chemokines IL-8, eotaxin-2, and TH2 attracting chemokines TARC and MDC and by costimulating mast cells to produce IL-5 and IL-13, as well as GM-CSF and IL-6. TSLP-activated mDCs mature and migrate into the draining lymph nodes to initiate the adaptive phase of allergic immune responses. TSLP-activated DCs express OX40L, which triggers the differentiation of allergen-specific naïve $CD4^+$ T cells to inflammatory TH2 cells that produce IL-4, IL-5, IL-13, and TNF but not IL-10. Inflammatory TH2 cells then migrate back to the site of inflammation, due to the local production of TARC and MDC. The TH2 cytokines IL-4, IL-5, IL-13, and TNF-α, produced by the inflammatory TH2 cells, initiate allergic inflammation by triggering IgE production, eosinophilia, and mucus production.

TABLE 1.1

TSLP induced DC maturation is uncoupled with IL-12 production

	TSLP-DC	CD40L-DC	TLRL-DC
CD80/CD86	Up	up	up
MHC II	Up	up	up
Survival	Up	up	up
IL-1α/β	–	++	++
IL-6	–	++	++
IL-12	–	++	++
IFNs	–	++	++
IP10	–	++	++
Eotaxin 2	++	–	–
IL-8	++	++	++
TARC (TH2)	++	–	+
MDC (TH2)	++	+	+