Review

Human TSLP-Educated DCs

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Thymic stromal lymphopoietin (TSLP), an IL-7-related cytokine, is widely expressed by epithelial cells in many tissues with different biological effects. Human TSLP (hTSLP) has been shown to play an important role in promoting T cell homeostasis, developing nondeletional central tolerance, amplifying epithelium-induced class switching, inducing atopic diseases and maintaining intestinal noninflammatory environment. Among diverse cells responding to hTSLP, dendritic cells (DCs) are the most obviously characterized target cells. In this review, we attempt to outline an effect of the functional versatility of hTSLP-activated DCs (hTSLP-DCs) on T cells. *Cellular & Molecular Immunology*. 2008;5(2):99-106.

Key Words: thymic stromal lymphopoietin, OX40 ligand, dendritic cell, T cell

Introduction

Dendritic cells (DCs), as a key linkage of innate and adaptive immunity, are the most professional antigen-presenting cells which can sense danger signals and prime naïve T cell immune response. Nevertheless, interaction of DCs with naïve T cells can also lead to T cell tolerance. It has recently become clear that mature DCs not only initiate primary immune responses, but also expanse peripheral regulatory T cells (Tregs). It's known that maturation of DCs can be induced by FLT-3 or GM-CSF *in vitro*, but how immature DCs undergo the maturation and the role of mature DCs in steady state *in vivo* is relatively unknown. Recent studies have introduced a novel and somewhat controversial brother of IL-7, hTSLP. It is expressed in human Hassall's corpuscles within the thymus, and drives the maturation of DCs in an antigen-independent manner with the capacity to instruct naïve T cells to differentiate into Tregs *in vitro*. For this reason, it is speculated that hTSLP-DCs might involve occurounce of naturally selecting Tregs. What's more, hTSLP-DCs can skew naïve T cells to Th2-biased phenotypes. More importantly, hTSLP is sufficient for initiating atopic diseases, such as atopic dermatitis and asthma, *via* the activation of two main sentinels, including DCs and mast cells which play a central role in host response to allergic challenge. Are we ready to pick hTSLP up as an important factor in immunity? Here, we try to integrate recent findings of hTSLP-DCs, as the principal hTSLP-responsive target, interacting with T cells into an emerging, although still fragmented, picture.

Cellular sources of hTSLP

TSLP was originally identified as a growth factor in culture supernatants of a thymic stromal cell line to support the development of murine immature B cells in 1994, which has recently been shown to be a key factor in B cell homeostasis (1, 2). However, few investigations on hTSLP had been made until hTSLP and hTSLP-specific chains (hTSLPR) were cloned by computational analyses of human genomic data in 2001 (3-6). hTSLP mRNA is mainly expressed by skin keratinocytes, epithelial cells (ECs), smooth muscle cells, lung fibroblasts or IgE/anti-IgE-stimulated mast cells but not IL-1/TNF-activated mast cells from *in vitro* studies (7-9). In the *in vivo* studies, hTSLP has also been shown to be expressed by endothelial cells, neutrophils and macrophages (10). Although hTSLP is highly expressed by keratinocytes in acute and chronic atopic dermatitis, it is undetectable in normal and non-lesional skin from patients with atopic dermatitis, or in skin lesions from patients with nickel-induced allergy contact dermatitis and cutaneous lupus erythematosus, or in the ECs from patients with Crohn disease, where high level of Th1 cytokine IFN-γ and transcriptional factor T-bet is produced, followed by inappropriate ratio of Th1 and Th2 (11-13).

The inducible expression of hTSLP is associated with several known pathogen-derived products, inflammation-related ligands and pro-inflammatory cytokines. When exposed to poly I:C and rinovirus, ECs release hTSLP, which can be synergistically and significantly enhanced by IL-4 (14-16).

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Table 1. Regulation of hTSLP production

Category	Stimuli	Regulation signals	References
Cytokines	TNF-α, IL-1β	p38, p42/p44, NF-κB	16, 17, 56
	IL-4, IL-13	ND	16, 18
	IL-1α, TGF-β, IFN-β	ND	16, 57
	IFN-γ	ND	58
Membrane protein	Fas ligand	ERK-1/2	20
	E-cadherin	ERK-1/2 and p38	19
Microbes	Flagellin, LPS, PGN	ND	9, 14, 58, 59
	Lipoteichoic acid, peptidoglycan	ND	59
	Klebsiella pneumoniae	ND	60
	Escherichia coli, S. typhimurium	ND	60, 61
	Lactobacillus plantarum	ND	59
Virus	RSVF protein	p38 and JNK	62
	dsRNA, rhinovirus	ND	16
	Poly I:C	ND	14
Others	Diesel exhaust particles	Oxidant-dependent manner	21
	Glucocorticoid	ND	16
	Dexamethasone	ND	58
	Physical injury	ND	9
To be determined in human	Water-soluble chitosan	ND	63
	Protease allergens	ND	64
	IL-33	ND	65
	Keratinocyte growth factor	ND	66
	Curcumin	ND	67
	Vitamin D3 and retinoic acid	ND	68

Meanwhile, LPS, PGN, CpGODN and IFN-α only marginally induce hTSLP production (14). If used alone, IL-4, IL-5 or IL-13 is not able to induce hTSLP production. Hewever, TNF-α or IL-1α synergizes with IL-4 or IL-13 to induce the expression of hTSLP (17). hTSLP is not affected by cytokines to regulate Th2 inflammation, such as IL-10, TGF-β and IFN-γ (18). Whereas, the treatment of 16HBEo-cells with IL-1β and 9-cis-retinoic acid (a ligand for retinoid X receptor, RXR) inhibits both basal and IL-1β-induced expression of hTSLP, which may be associated with the repression of NF-κB activation by RXR (17). Down-regulation of E-cadherin leads to up-regulation of hTSLP, whereas up-regulation of Fas ligand increases hTSLP production (19, 20). Besides, air pollutants like diesel exhaust particles can also induce hTSLP expression (21). Thus, viral infection or allergens may recruit pro-allergic cytokines-producing cells, which would in turn augment the expression of hTSLP on bronchial airway epithelium (Table 1).

The NF-κB signaling which can be triggered by various bacterial, viral products and trauma as well as cytokines has been shown to play an important role in innate immunity and adaptive immunity. Li M, et al. showed that selective RXRα and RXRβ ablation in the keratinocytes led to rapid increase of mouse TSLP (mTSLP) expression, which may be explained by competitive recruitment of transcription integrators between NF-κB and RXR (22, 23). Another study showed that deletion of IKK-β resulted in a reduced expression of mTSLP in intestine ECs, providing direct evidence that NF-κB is involved in inducing mTSLP expression (24). In human, hTSLP is only expressed in the lesional skin from patients with atopic dermatitis, as various forms of cutaneous and mucosal trauma call up a cascade of celluar and molecular events mediated by NF-κB (8, 25). The promoter region of mTSLP/hTSLP gene includes NF-κB-binding sites, and the region between -4.0 and -3.7 kb of hTSLP gene promoter contains cis-regulatory elements involved in inducible expression of hTSLP. What's more, p65/p50 binds to the NF-κB motif in the promoter in IL-1β-treated A549 cells. In reciprocal experiments, mutation of the NF-κB motif in -3.86 to -3.74 kb of the promoter significantly decreases its activity, and over-expression of a dominant-negative version of IKK-β abolishes hTSLP promoter activation (17). Since the expression of hTSLP controlled by NF-κB is essential for immune homeostasis, the precise inside-out and outside-in signaling regulation of inducible and intrinsic hTSLP expression needs a closer look.

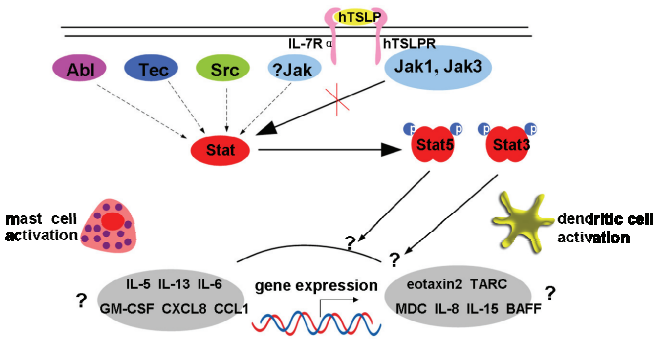

Figure 1. Activation of DCs and mast cells. Engagement of hTSLP with its receptor complex comprised of IL-7Rα and hTSLPR heterodimer results in Stat5 and Stat3 phosphorylation. However, the phosphorylation of Stat is not mediated by recruitment of Jak1 or Jak3. It is suggested that there may be a novel Jak or other tyrosine kinases responsible for Stat activation.

hTSLP-mediated signaling

It has been confirmed that the absolute requirement for heterodimerization of the IL-7Rα and IL-7Rγc decides to activate IL-7-specific signaling events (26). Similarly, the functional receptor complex for hTSLP is a heterodimer that consists of hTSLPR (that is closely related to IL-7Rγc) and the IL-7Rα. Following binding of hTSLP to hTSLPR/IL-7Rα, one of the key events is the activation of Stat5 and Stat3 (3). However, although the receptors for hTSLP and IL-7 share the common component of IL-7Rα, as well as they all induce the phosphorylation of Stat5, hTSLP does not activate Jak1 and Jak3, indicating that hTSLP and IL-7 activate Stat5 *via* distinct signals (3, 6, 7, 27). Consistent with this, none of the four known Jaks are activated in response to cytokine-mediated stimulation of mTSLP receptor, but Tec kinase family member Tec may involve TSLP-mediated Stat5 activation (6, 28, 29). Notably, erythropoietin (EPO) induces the tyrosine phosphorylation and activation of Jak2 and Stat5 when hTSLPR cytoplasmic domain is fused with erythropoietin receptor (EPOR) (27). Like Stat5, it is well established that Stat3 is activated by tyrosine phosphorylation through Jaks or Src family kinases in response to various cytokines or growth factors. However, it remains unknown how hTSLP utilizes Stat5 and Stat3 to prime target cells. Is there any novel Jak or other tyrosine kinases such as Src, Tec or Abl kinases responsible for activation of Stat5 and Stat3? Several cell types including activated DCs and monocytes produce significant levels of both hTSLPR and IL-7Rα although it is unknown about what transcription factors orchestrate their expression (3). hTSLP has so far been discovered to have an influence on DCs, mast cells and activated T cells, and there is somewhat controversy about its influence on B cells (30) (Figure 1). Instead, mTSLP may exert its activity primarily on B cells, T cells and to lesser extent on DCs (2, 7, 9). Moreover, mTSLP and hTSLP share only 43% of amino acid identity and there has no cross-reactivity between mTSLP and hTSLP receptor complex (3, 5). Here, our interest in hTSLP focuses on its function on DCs.

Cytokine profiles of hTSLP-DCs

hTSLP has been found not only to strongly activate DCs and langerhans cells (LCs), but also to maintain their survival, as a result of activation of Stat5 and Stat3 (7, 31, 32). However, unlike CD40L or TLR ligands, hTSLP-DCs do not produce Th1 cell-polarizing cytokines including IL-12, IL-23, IL-27, IFN-γ, IFN-α, IFN-β, pro-inflammatory cytokines such as IL-1β, IL-4, IL-6, IL-13, TNF-α, anti-inflammatory IL-10 or IP10/CXCL10, as well as monokines induced by IFN-γ (7, 8, 33). In contrast, hTSLP-DCs produce high levels of Eotaxin2, TARC and MDC as well as IL-8 and IL-15, which favors recruitment of Th2 cells toward inflammatory sites and reflects the disease activity in patients with asthma (7, 8, 10). Furthermore, hTSLP-DCs also up-regulate constitutive expression of membrane bound BAFF and soluble BAFF to support immature B cell to survive, mature, and to augment intraepithelial class switch (8, 14). Notably, hTSLP can strongly enhance expression of IL-12 and co-stimulatory molecules including CD40, CD80 and CD86 by CD40L-activated DCs, which corresponds to TCR signal strength correlated with IL-12 production (34, 35). Moreover, T cells primed by hTSLP plus CD40L conserve their ability to produce high levels of IL-4, IL-5, IL-13 and TNF-α in the presence of IL-12 due to integrated effects of cytokine regulation, co-stimulatory molecule interactions and TCR signaling strength (34). However, the cytokines produced by hTSLP-DCs in physiological settings are still unclear. Rimoldi et al. proposed that EC-conditioned DCs with physiological amounts of hTSLP drove non-inflammatory Th2 but not Th1 polarization even after exposure to *S. typhiumurium* (36), which, together with the finding that hTSLP is constitutively expressed by colon epithelium but undetectable in patients with Crohn disease, suggests that hTSLP is crucial for maintaining gut homeostasis. Further study to quantify the dose of hTSLP used is critical for EC-conditioned DCs to release IL-12, which is released only within a narrow 'window' of hTSLP (0.07-0.15 ng/ml). Altogether, these data suggest that homeostatic expression of hTSLP leads to non-inflammatory DCs, whereas deregulated expression of hTSLP drives inflammatory DCs.

Interactions between DCs and T cells mediated by hTSLP

hTSLP activates both mDCs and Langerhans cells (LCs) with increased expression of CD83, CD86, OX40 ligand (OX40L) and MHC II, endowing them with powerful capacity to cause the expansion and differentiation of different T cell subtypes. In the presence of exogenous antigen, hTSLP-DCs induce allogeneic naïve T cells turning into pro-allergic T cells with the increase of IL-4, IL-5, IL-13 and TNF-α, and the

decrease of IL-10 (8, 31). It has been known that LPS may induce Th1 responses depending on the TLR adaptor MyD88 and Th2 responses in MyD88 deficient mice (37). However, hTSLP primes mDCs with the capacity to skew Th2-bias responses through a unique signaling pathway that is dependent on Stat5 but independent on classical NF-κB and MyD88 signaling pathways (7, 33). The signals from hTSLP-DCs are the most effective on up-regulation of the genes involved in Th2 polarization, such as CRTH2, GATA-3, c-MAF and IL17RB (38). Expression of OX40L on the surface of hTSLP-DCs primes $CD4^+$ T cells by the high level of GATA3, c-Maf and the low level of T-Bet to generate TNF-α^+IL-10^- inflammatory Th2 cells concomitantly producing IL-4, IL-5 and IL-13 (33, 39). As observed, anti-OX40L or anti-IL-4 mAb strongly inhibited the production of IL-4, IL-5 and IL-13, followed by the great reduction of GATA3 and c-Maf expression, and the combination of both almost completely switched a Th2 response to a Th1 response primed by hTSLP-DCs (33). Consistent with this, adding IL-12 into the $CD4^+$ T cells that were primed by hTSLP-DCs induced inflammatory Th1 responses by strongly inhibiting GATA3 and c-Maf, and increasing T-Bet expression (33, 40). Conversely, hTSLP was sufficient to reduce endogenous IL-12 expression (36). Anti-OX40L mAb dramatically inhibits TNF-α production, but promotes IL-10 production by $CD4^+$ T cells primed by hTSLP-DCs (29). OX40L also converts an IL-10-producing regulatory Th1 response induced by IL-12 into a TNF-α-producing inflammatory Th1 response (33). Similar to this, OX40L inhibits the generation of IL-10-producing Tr1 cells from naïve and memory $CD4^+$ T cells induced by immunosuppressive drugs, such as dexamethasone and vitamin D3, the inducible co-stimulatory ligands and immature DCs (41). In addition, hTSLP-DCs can strongly expand $CD8^+$ T cells with high expression of IL-5 and IL-13, and induce cytotoxic $CD8^+$ T cells in the presence of CD40L, which may also be regulated by OX40L (42, 43). The evidence implicates that OX40L expressed on the surface of hTSLP-DCs drives naïve T cells to differentiate into pro-allergic Th2 cells by competing with production of IL-12. However, this conclusion is challenged by the studies using LCs, as no differences of OX40L expression were found on hTSLP-treated and -untreated migratory LCs (31). Therefore, it remains obscure whether OX40L is necessary for hTSLP-LCs to induce inflammatory T cells. Actually, TSLP is able to direct human $CD4^+$ T cell differentiation *via* DCs. It can also directly drive Th2 differentiation in the absence of exogenous IL-4, which is dependent on Stat6 and independent on IL-2. The fact that the TSLP treatment leads to direct IL-4 gene transcription suggests that TSLP was involved in Th2 differentiation *via* the induction of endogenous IL-4 production (54).

In the absence of exogenous antigens, hTSLP-DCs strongly induce polyclonal expansion of autologous naïve $CD4^+$ T cells without additionally skewing naïve T cells toward a pro-allergic Th2 phenotype (35). Under this condition, OX40L/OX40 interaction between DCs and T cells contributes to hTSLP-DC-treated $CRTH2^+CD4^+$ memory T cell longevity, which may be associated with the induction of Bcl-xL and Bcl-2 expression (38, 44, 45). Homeostatic proliferation of this T cell population is supported by hTSLP-DCs and after multiple rounds of stimulation, they maintain the central memory T cell phenotypes and Th2 commitment (38). Further investigation showed that naïve $CD4^+$ T cells activated by autologous hTSLP-DCs acquire the central memory phenotypes with high CD62L expression, down-regulated CD45RA and up-regulated CD45RO, producing large amounts of IL-2, but little IL-4, IL-5, IL-10 and IL-13 after re-stimulated with anti-CD3 and anti-CD28 (46). This similar function to IL-7 in memory $CD4^+$ T cells may partly explain why memory $CD4^+$ T cells lacking γc exhibit normal homeostatic proliferation and antigenic responses. In addition, IL-17E promotes human Th2 memory cell expansion with Th2 cytokine production and augments the functions of the Th2 memory cells when they are stimulated with hTSLP-induced DCs. The enhanced functions of the Th2 memory cells by IL-17E are associated with sustained expression of GATA-c-MAF and JunB in an IL-4-independent manner. Elevated expression of IL-17E and IL-17ER transcripts has been found in asthmatic lung tissues and atopic dermatitis skin lesions, exerting possible roles in exacerbated allergic disorders (55). In conclusion, hTSLP-DCs promote $CD4^+$ T cell homeostasis by expansion of autologous naïve $CD4^+$ T cells and by tweaking $CD4^+$ memory T cell phenotypes, which may be dependent on the greatly enhanced expression of self peptide-MHC complexes and co-stimulatory molecules, especially OX40L on DCs that sustains DCs and T cell conjugate formation and prolongs DC survival.

For $CD4^+CD25^-$ thymocytes, hTSLP-DCs are capable of instructing them to differentiate to $CD4^+CD8^-CD25^+$ Tregs with Foxp3 mRNA expression at a level similar to the Foxp3 mRNA level in natural Tregs *in vitro* (47). Additional support to this study proved that mTSLP increases expression of Foxp3 as well as $CD4^+CD8^-CD25^+$ Tregs in FTOC (fetal thymus organ culture) model, and the expression is inhibited by blocking mTSLPR (48). However, TSLPR-deficient mice do not exhibit any differences in Treg development (7). One explanation of this phenomenon is due to species differences, as Hassall's corpuscle is well developed in the thymus of human but poorly of mice. Another explanation is due to redundant roles of hTSLP in positive seletion of Tregs. The pleiotropic functions of hTSLP-DCs on different T cell subsets are described in Figure 2.

Uncoupling of hTSLP-DCs from TECs in Treg development

It is well known that ECs in the thymic cortex mediate dominant tolerance, whereas DCs in the thymic medulla mediate negative selection to purge useless T cells. hTSLP is expressed in Hassall's corpuscle where $CD11c^+$ DC-$LAMP^+$ activated DCs localize and $CD4^+CD25^+$ Tregs are found exclusively in the medulla. Thus, DCs in the medulla might involve selection of Tregs and hTSLP-DCs function to instruct Treg differentiation (7). It is proposed that developing

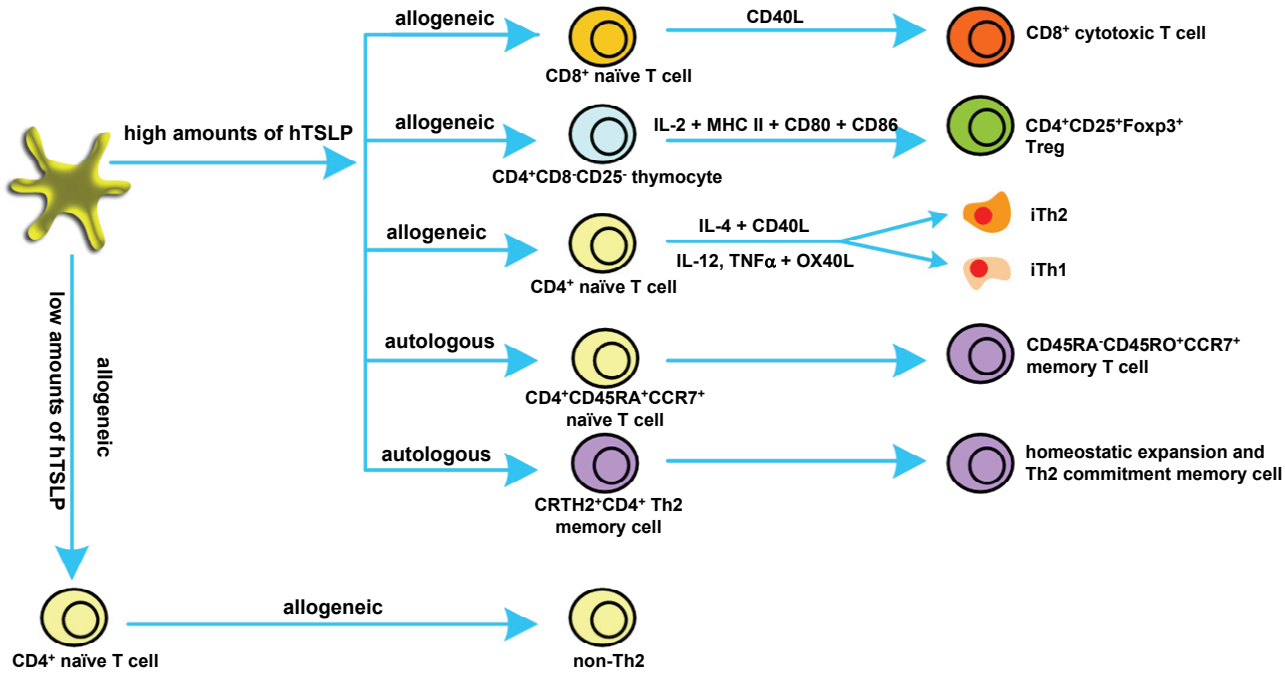

Figure 2. Roles of hTSLP in DC-T cell interaction. A large amount of hTSLP induces allogeneic naïve T cells to differentiate into cytotoxic T cells or inflammatory Th1/Th2 cells, convert single positive thymocytes Tregs, drive autologous naïve T cells to differentiate into memory T cells and maintain autologous Th2 memory T cell phenotypes. In contrast, small amounts of hTSLP promote non-inflammatory classical Th2 cell differentiation.

thymocytes undergo three types of selection once mature TCR complex is expressed: primary positive selection, negative selection, and secondary positive selection that leads to nondeletional central tolerance. Regretfully, there is no direct evidence that hTSLP induces DC maturation, thus convert high-affinity thymocytes to Tregs *in vivo*. If the notion of hTSLP-DCs mediating Treg development is correct, it is likelihood that hTSLP-DCs may be separated from thymic ECs (TECs) with function to select Tregs. We reason that this case may be based on following considerations: 1) Immune system is adept at using the same signaling to play dual roles in DC-T cell interactions. 2) Tregs play a dominant role in self-tolerance. 3) Self-antigens on TECs might be cross-presented by DCs. 4) hTSLP-DCs can induce single positive thymocytes to differentiate into Tregs. 5) DCs may not only purge some harmful Tregs, but also select useful Tregs in the thymic medulla.

Atopic diseases: err on the side of caution?

Atopic diseases are associated with a genetic predisposition, which are caused by dysregulated Th2-biased immune responses. Compelling evidence suggests that hTSLP is at the top of a complex immunological cascade that leads to atopic dermatitis and asthma. hTSLP-DCs can induce the differentiation of T cells into pro-allergic effectors with large mounts of cytokines IL-4, IL-5, IL-13, TNF-α and little IL-10. hTSLP in keratinocytes from patients with atopic dermatitis appears to trigger epidermal Langerhans cells to migrate from epidermis into dermis to induce allergic-specific T cells (31). The increased hTSLP in airway epithelium stimulated by pro-inflammatory mediators or from patients with asthmatics correlates with expression of Th2-attracting chemokines and disease severity (10). Apart from being a primer to allergic inflammation, hTSLP also contributes to long-term atopic diseases. As observed, hTSLP-DCs maintain $CRTH2^+$ Th2 central memory phenotypes and Th2-biased cytokine profiles. Furthermore, hTSLP-DCs induce $CRTH2^+$ Th2 to express some genes encoding cystain A, Charot-Leydon crystal protein, and prostaglandin D_2 synthase expressed uniquely by eosinophils and basophils, which further underscores the role of hTSLP in the allergic inflammation (38). Additionally, mast cells, as tissue-dwelling effectors, play a crucial role in allergic diseases producing high levels of IL-5, IL-13, IL-6, GM-CSF, CXCL8 and CCL1 when activated by hTSLP in the presence of IL-1 and TNF (9). Collectively, hTSLP empowers DCs and mast cells to initiate atopic diseases. However, in the evolutionary battle against infectious diseases, it seems unlikely that hTSLP has evolved to facilitate atopic diseases, as Th2-associated immunity is also a key player in protecting us from parasites invading. What are the physiological effects of homeostatic expression of hTSLP? As mentioned above (36), physiological amounts of hTSLP render DCs to drive non-inflammatory Th2 and counteract the potential inducible Th1 response, indicating that hTSLP also involves the protection from Th1-induced diseases. Therefore, homeostatic expression

of hTSLP is speculated to play a critical role in Th1 responses.

Conclusions

hTSLP can be released by human ECs in response to microbes, physical injury and pro-inflammation cytokines, which is regulated by NF-κB signaling. It is clear that hTSLP primed-DCs are dependent on activation of Stat5 and Stat3 with increased expression of CD83, CD86, OX40L and MHC II. hTSLP-DCs have the capacity to promote T cell homeostasis, induding expanding both autologous and allogenic naïve T cells, tweaking $CD4^+$ T memory cell phenotypes, inducing Treg commitment from thymic $CD4^+$ T cells, inducing pro-allergic T cells to play a prominent role in atopic diseases. Additionally, hTSLP is capable of activating mast cells to produce Th2-biased cytokines, providing a non-T cell route to initiate atopic diseases. Thus, hTSLP represents a promise therapeutic target for treatment of human atopic diseases, which might be reconciled by fine-tuning hTSLP-induced cytokines, such as IL-1β and TNF-α and by regulating hTSLP-responsive cells, such as DCs and mast cells through OX40L or other factors. Besides, several critical questions remain unanswered. What are the upstream and downstream signals of Stat5 and Stat3 in regulating hTSLP-DCs? What is the precise role for OX40L in hTSLP-DCs? Why and how do hTSLP-DCs instruct Treg commitment selectively in single positive thymocytes?

Emerging data lend supports to the dynamic conversion between T lineages by cytokines, transcription factors, immune suppressors, cell-cell contact and so on. The physiological role of hTSLP-DCs may extend to peripheral tolerance. It is because peripheral tolerance might be broken down in allergic and asthmatic diseases due to the maladjustment of hTSLP. In addition, 'tolerogenic' DCs have been implicated in the induction of 'extrathymic' Tregs and Tregs can be expanded by mature DCs both *in vitro* and *in vivo* (49-51). Thus, hTSLP should be a promising candidate priming DCs to instruct Treg commitment from peripheral naïve $CD4^+$ T cells or to expand Tregs probably by synergizing with IL-2, IL-10, TGF-β, thrombospondin-1 and Jagged (37, 52, 53). Given the broad roles of hTSLP-DCs in T subpopulation and their putative role in central and peripheral immune tolerance, it would be necessary to further explore biological effects of hTSLP-DCs on different T subsets or other cells.

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