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Thymic Stromal Lymphopoietin (TSLP) and Cancer

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Abstract

Originally shown to promote the growth and activation of B cells, thymic stromal lymphopoietin (TSLP) is now known to have wide-ranging effects on both hematopoietic and non-hematopoietic cell lineages. These include dendritic cells (DCs), basophils, mast cells, $CD4^+$, $CD8^+$ and natural killer (NK) T cells, B cells and epithelial cells. While TSLP's role in the promotion of TH2 responses has been extensively studied in the context of lung- and skin-specific allergic disorders, it is becoming increasingly clear that TSLP may impact multiple disease states within multiple organ systems. This review will highlight recent advances in the understanding of the surprising role of TSLP in the control of a variety of cancers, both solid tumors and leukemia, where the TSLP/TSLPR axis has been shown to be an important regulator.

TSLP Biology

Thymic stromal lymphopoietin (TSLP) is a member of the 4-helix bundle cytokine family, and a distant paralog of IL-7(1). As the name suggests, TSLP was first identified as an activity in the supernatants of a mouse thymic stromal cell line that was capable of supporting immature B cell proliferation and development(2-4). In addition, TSLP could act as a co-stimulator for thymocyte proliferation, suggesting that it acted as a lymphopoietin(1). A TSLP homolog was subsequently identified in humans using *in silico* methods(5,6). Similarly, several groups isolated a TSLP-binding protein in both humans and mice (referred to as TSLP receptor (TSLPR)), which bound TSLP with low affinity(7-10). Sequence analysis found that TSLPR was most closely related to the common gamma chain (γ_c; (7)). It is now known that the functional, high affinity, TSLPR complex is a heterodimer of TSLPR and interleukin 7 receptor alpha (IL-7Rα)(7,8). Cross-species homology for both the cytokine and its receptor is relatively low (~40% for each), although, as described below, functionally they appear to be quite similar. Thus, the role of this cytokine axis is conserved between man and mouse in spite of a loss of sequence identity.

The similarity of TSLP to interleukin (IL)-7, and the homology of TSLPR to γ_c suggested that TSLP may play a role in regulating lymphocyte development and/or function. Indeed, early studies did show that TSLP was capable of influencing both T and B cell development and proliferation, both *in vitro* and *in vivo*(1,4,11). However, the effects were modest suggesting that the influence of TSLP on lymphocyte development is redundant.

Analysis of the expression profiles of the two receptor subunits in human cell populations provided important insights into the primary biological role of TSLP. The cell population with the highest known coexpression of TSLPR and IL-7Rα were myeloid dendritic cells (DC) (5). Confirming the expression data, TSLP treatment of human DCs induced several

phenotypic changes, including increased survival, upregulation of major histocompatibility complex class II (MHCII) and co-stimulatory molecules (CD86 and CD40), and production of a variety of chemokines, most notably the CCR4 ligands CCL17 and CCL22(5,12). Murine bone marrow-derived DCs acquired a similar activated phenotype following TSLP stimulation(13).

Little is known as to the signaling pathways that are activated following engagement of the TSLP receptor complex. Initial studies in the mouse showed that signal transducers and activators of transcription (STAT)5 was activated, but in the absence of detectable JAK activation(2), making TSLPR unique among members of the hematopoietic receptor family. However, two subsequent papers have demonstrated robust and sustained activation of JAK-1 and -2 following TSLP signaling in primary human dendritic cells and primary human and mouse CD4+ T cells(5,14). Surprisingly, unlike IL-7Rα and γc in IL-7 signaling, which utilize JAK-1 and -3, the TSLPR subunit bound and utilized JAK-2 in concert with IL-7Rα-associated JAK-1. These findings resolve a long-standing question about the mode of TSLP signaling, and show that TSLP-induced JAK activation precedes the activation of STAT proteins. In the human, studies have shown that, in addition to STAT5, TSLP stimulation activated STAT 1,3,4, and 6, as well as JAKs 1 and 2(15). One possible explanation for the discrepancy in the data between species is that the mouse signaling work primarily used a pre-B cell line, while the human studies were largely in primary dendritic cells. Consistent with this explanation, our lab has shown that TSLP-treated mouse DCs activate Jak1 and Jak2, as well as STATs 1, 3, and 5 although only Stat5-deficient DCs fail to induce TSLP-specific genes(16). In addition, studies using non-hematopoietic cells (airway smooth muscle cells) have shown that TSLP signals through Stat3(17). Taken together, these data demonstrate that TSLP is capable of activating multiple STAT proteins. Whether TSLP utilizes similar signaling pathways in other cell lineages and how each STAT molecule contributes has yet to be elucidated.

As mentioned above, several cell types have been shown to respond to TSLP. TSLP was originally isolated and characterized as a lymphocyte growth factor(1,2,4), and subsequent studies have shown that TSLP can promote T cell proliferation and differentiation both in vivo and in vitro(18-20). Finally, as will be detailed below, TSLP responsiveness of CD4 T cells is a critical feature of the challenge phase of allergic inflammation(21,22).

It has now become apparent that a major TSLP-responsive cellular subset in both humans and mice are myeloid-derived dendritic cells (mDCs)(5,13). Co-culture of TSLP-activated DCs with naïve syngeneic $CD4^+$ T cells led to T cell proliferation but no differentiation, suggesting a role for TSLP in $CD4^+$ T cell homeostasis(23). However, when TSLP-stimulated DCs primed $CD4^+$ T cells in an antigen-specific manner (e.g., in an allogeneic culture), the resulting T cells display characteristic features of Th2 differentiated cells (production of IL-4, IL-5, IL-13, and TNFα), with the exception that IL-10 production was not evident(12). These data suggest that TSLP-activated DCs primed for inflammatory Th2 cell differentiation. Interestingly, TSLP, in the absence of IL-12, induced OX40L expression on DCs, and OX40-OX40L interactions were critical for the ability of the DCs to drive Th2 cell differentiation(24). Consistent with a role in regulating Th2 cytokine responses, TSLP-activated DCs were also capable of supporting the maintenance and further polarization of

Th2 effector memory cells(25). TSLP-conditioned DCs also augmented intestinal epithelial cell-mediated IgA2 class switching through the induction of APRIL (21). Finally, some *in vitro* studies have suggested a role for TSLP in the generation of tolerogenic DCs that can drive the differentiation of regulatory T cells (Tregs) (26-28), although other studies have indicated that TSLP may hinder the production and/or maintenance of FOXP3+ Tregs *in vivo* in certain disease processes (29).

Finally, several innate immune cells express the TSLPR and respond to TSLP. For example, TSLP can enhance cytokine production from mast cells, NKT cells and eosinophils (30-32). Recent work has highlighted direct effects of TSLP on basophils during TH2 cytokine-associated inflammatory diseases, including promotion of basophil hematopoiesis from the bone marrow in an IL-3-independent manner (33).

Taken as a whole, the plethora of cell types that can respond to TSLP demonstrate the important role of this cytokine in orchestrating the initial response to an epithelial insult (Figure 1). While the normal function of TSLP is likely the maintenance of Th2-type homeostasis at barrier surfaces(14), dysregulated TSLP expression can result in the development of type 2 inflammatory responses leading to allergic disease.

Recently a new and unexpected function for TSLP has been found for the induction and regulation of a variety of tumors. TSLP has been found to both promote and suppress solid tumor growth, and somatic mutations and chromosomal translocations in genes encoding members of the TSLP receptor complex have been found in a subset of pediatric patients with B cell acute lymphocytic leukemia (B-ALL). The remainder of this review will discuss this aspect of TSLP biology, along with the potential for therapeutic intervention through modulation of the TSLP pathway.

Role for TSLP in growth and metastasis of solid tumors

It has been shown that for many different types of cancers, a Th2 response is dominant over cytotoxicity induced by CD8 T cells and T-helper 1 (Th1) response(34). Tumors with this type of phenotype generally have a worse prognosis relative to tumors where Th1-type responses predominate(35,36). However, the mechanism by which Th2-biased immune responses are initiated in tumors remains largely unknown. However, two recent studies in humans demonstrated a role for TSLP in promoting a Th2-like environment in the tumor through expression of the cytokine in the tumor microenvironment (Fig. 1). In the first study, De Monte et al.(36), studying pancreatic cancer where a GATA3+ Th2 cellular infiltrate is dominant, showed that cancer associated fibroblasts (CAFs) can produce TSLP. In vitro, supernatants from CAFs were capable of activating DCs to drive Th2 differentiation. Importantly, they found that tumors and tumor-draining lymph nodes contained TSLPR+ DCs, while non-draining lymph nodes did not(36). Finally, using a completely in vitro system, TSLP was shown to be released by human cervical carcinoma cells(37). These authors suggested that this tumor-derived TSLP can act on TSLPR+ endothelial cells to promote angiogenesis in cervical cancer. These data suggest that there is cross-talk between hematopoetic cells that infiltrate the tumor and stromal elements

associated with the tumor that can promote a microenvironment favorable to the tumor itself.

In the second study Pedroza-Gonzalez et al.(38) investigated the factors that drive a Th2 microenvironment in breast tumors. They demonstrated that TSLP is produced directly by tumor cells in breast cancer patients. They found that supernatants from explanted tumors were capable of inducing OX40L on DCs in a TSLP-dependent manner, and that these DCs could then promote Th2 differentiation of naïve CD4 T cells. In addition, OX40L+ DCs were found in breast tumors. Interestingly, they used a xeno transfer model to show that blockade of either TSLP or OX40L could reduce tumor growth and IL-13 production(38). Taken together, these 2 papers suggest that TSLP is an important player in promoting tumor survival through manipulation of the immune response in the tumor itself, and that TSLP blockade could be an important therapy for these cancers.

The role of TSLP in tumor growth and metastasis is supported by work using an orthotopic model of metastatic breast cancer in the mouse(39). This model uses a cell line (4T1) derived from a Balb/c breast ductal carcinoma, which, when transplanted into a mammary gland leads to growth of primary tumor with metastases to several organs, including lung(40). This group showed that 4T1 cells produce TSLP, and that the level of TSLP expression is correlated with tumor growth and metastasis. They also found that primary tumor growth was delayed when transplanted into TSLPR-deficient hosts. Unlike the studies in human tumors, they found that the deficit in these mice was not due to lack of TSLP responses in DCs, but rather it was $CD4^+$ T cells that required TSLP responses. Our lab has found that transplantation of 4T1 cells into a TSLP-deficient host results in strikingly reduced growth of the primary tumor and a complete inhibition of lung metastasis (Kuan and Ziegler, in preparation). While the underlying mechanism is not yet clear, we have found that TSLP can functionally activate monocytic lineage myeloid-derived suppressor cells, and that they are lacking in the tumor bearing TSLP-deficit hosts.

In contrast to these studies that suggest a tumor-promoting role in TSLP, two independent groups demonstrated a tumor-suppressing role in TSLP in murine model of skin cancer(41,42). Both papers used keratinocyte-specific ablation of Notch signaling, which has been shown to lead to skin barrier defects and TSLP-dependent dermatitis(43). Demehri et al. showed that mice with inactivation of Notch signaling through deletion of RBPj in keratinocytes failed to develop skin tumors, even following a chemical carcinogenesis regimen that lead to tumor formation in wild-type mice. They found that blocking TSLP signaling in these mice reduced dermal inflammation and allowed for tumor formation, and that induction of TSLP in the skin of wild-type mice inhibited tumor formation. Using a variety of techniques they found that TSLP-responsive CD4 T cells were both necessary and sufficient for the effects of TSLP in this model.

Using a similar strategy, De Piazza et al.(41) found that induced deletion of Notch1 and Notch2 in keratinocytes leads to development of dermatitis and hair follicle associated cysts. Deletion of TSLP signaling in these animals led to overt tumor formation. Unlike the previous study, they showed $CD8^+$ T cells, but not $CD4^+$ T cells, NK cells, B cells, or DCs, are more important for TSLP suppression of tumor formation(41). This group also indicated

TSLPR signaling may function differently in distinct cell types. For example, TSLP signaling in $CD11b^{+}Gr1^{+}$ cells, which are generally viewed as granulocytic myeloid-derived suppressor cells(44), in this model are tumor-promoting instead of tumor-suppressing. A better understanding of the complexity of TSLP-responsive cell subsets in the context of tumors is clearly required to sort out these data. Another interesting concept in the Demehri et al. paper is that the temporal and magnitude of TSLP expression is critical(42). Interestingly, a recent study using the 4T1 transplant breast tumor model showed that transplanted TSLPR deficient mice displayed greater metastasis to the brain but lower in lung(45). This paradoxical result, they claimed, may be because the blood brain barrier keeps tumor cells out. But enhanced systemic Th1 responses they observed in TSLPR deficient tumor bearing mice may open this gate for tumor cell entry. TSLP, although not detected in normal skin, is expressed in glandular breast epithelial cells in non-tumor normal donors.

TSLP receptor complex and Pediatric B-ALL

Pediatric acute lymphocytic leukemia (ALL) is a very heterogeneous disease that is associated with a variety of genetic lesions, including recurring chromosomal translocations, deletions and amplifications(46). It is the most common childhood tumor, and while 80% of affected children are successfully treated, it remains a leading cause of childhood morbidity and mortality(47,48). Recent advances in molecular genetic profiling of B-ALL have uncovered the nature of many of these genetic abnormalities. They include chromosomal translocations (e.g., ETV6-RUNX1, BCR-ABL and TCF3-PBX1) and mutations in genes known to be involved in B cell development (e.g., PAX5, EBF1 and IKZF1)(46). The IKZF1 mutations are especially interesting in that they are a hallmark of Philadelphia chromosome (Ph+) B-ALL (with BCR-ABL translocations) with poor outcomes, but are also seen in Ph− cases that resemble Ph+ patients (referred to as Ph-like ALL)(49,50). These Ph-like cases encompass approximately 15% of B-ALL, and have a higher risk of relapse when compared to Ph+ cases .

Using a variety of methods several groups simultaneously found that mutations in the TSLP signaling pathway correlated with a significant number of B-ALL cases (Fig. 2). For example, ~50% of the Ph-like patients were found to have chromosomal rearrangements involving the TSLPR gene (also referred to as CRLF2)(51). These rearrangements include deletions that join TSLPR and P2RY8 (a gene closely linked to *TSLPR*) and translocations between TSLPR and the IGH locus(51-53). These alterations lead to increased expression of the TSLPR by coupling its expression to the promoter/enhancer of the translocation partner. In addition, these translocations were also seen in approximately 60 percent of acute lymphoblastic leukemia cases in children with Down's Syndrome(52-54). Interestingly, these mutations were highly correlated with the presence of JAK2 mutations and were associated with a poor prognosis(55-57). Thus, genetic alterations in TSLPR gene expression are associated with a form of B-ALL with poor prognosis.

In addition to the chromosomal rearrangements, an activating mutation in the TSLPR gene has also been found in Ph-like B-ALL. This mutation changes a Phenylalanine residue in the extracellular domain of the TSLPR adjacent to the transmembrane domain to a Cysteine

(F232C)(58). This leads to a gain-of-function as the resulting TSLPR is able to constitutively homo-dimerize and signal. Interestingly, a similar mutation in the IL7Rα, which forms a heterodimer with TSLPR to generate a functional TSLP receptor complex, has been found in B-ALL (S185C)(59). In addition, insertions and deletions within the transmembrane domain of the IL-7Rα, all of which resulted in the presence of a *de novo* Cysteine residue, were found in several patients(59). Finally, activating mutation in JAK2 have been associated with elevated TSLPR expression in B-ALL(60). These data make a compelling case for enhanced TSLP receptor signaling and the development of B-ALL.

The predominant method that has been used to assess the functional consequences of mutations in TSLPR/CRLF2, IL-7Ra and JAK2 has been expression in factor-dependent cell lines. Retroviral transduction of the mutant genes into the factor dependent cell line BaF3 showed that their expression rendered the cell line growth factor independent(51,58,59,61). While these experiments provided insights into the consequences of these mutations on signaling from the receptor, they did not address the nature of cell affected in vivo or the effect on overall B cell development driven by these mutations.

In an attempt to determine the in vivo function of the TSLPR/CRLF2 F232C mutation, bone marrow of wild-type mice was transduced with retroviruses cDNA clones expressing the human mutant, followed by transplantation into lethally-irradiated hosts. From these studies one animal displayed splenomengaly and indications of increased myeloproliferation in the blood, and elevated number of immature granulocytes and megakaryocytes in the bone marrow(58). While this study demonstrates that TSLPR F232C is an activated allele, there are important issues with these studies. First, the authors stated that the transduced bone marrow progenitors did not contribute to the lymphoid compartment in an appreciable manner, thus limiting the usefulness of this approach for studying B-ALL. Second, the method used to introduce the mutant receptor allows its expression in all hematopoietic lineages. This may allow for off-target effects as other studies suggest that the mutations occur somatically(51,59). Finally, and possibly most important, using the human TSLPR for these studies precludes the ability to determine whether the mutated receptor requires interaction with IL-7Rα and subsequent binding to TSLP as the human and mouse cytokines are species specific in their ability to bind the TSLP receptor complex(7). While these studies are important in that they demonstrate altered functionality of the mutant protein, they have limitations.

Mice with systemic over-expression of TSLP may provide a model for understanding the signaling mechanisms involved. Interestingly, over-expression of TSLP early in the postnatal period was sufficient to drive a B cell lymphoproliferative disorder, but administration or induction of TSLP after postnatal day 14 was not, although other studies have shown expansion of B cell compartments following TSLP expression in adult mice(62). Importantly, in these studies the target of TSLP in the bone marrow were late pro-B cells, similar to the phenotype seen in pediatric B-ALL(62). One possibility is that the acquisition of mutations targeted to the TSLP signaling pathway leads to an unregulated expansion of this population of B cell progenitors, allowing for subsequent neoplastic transformation. The development of appropriate animal models is required to properly test whether this is the case.

Conclusions

A role for TSLP in type-2 inflammatory responses, especially those at barrier surfaces, is now accepted. In the past 4-5 years a new role for TSLP in tumor immunology has emerged. Interstingly, these studies have found a rather complicated role for TSLP, with being tumor-promoting in some instances and tumor-inhibiting in others. Furthermore, enhanced activation of the TSLP signaling pathway can lead to neoplastic transformation of B cell progenitors. Therefore, the decision how to manipulate TSLP or its signaling pathway is dependent on the tumor type. Defining more specific target(s) underling TSLPR signaling that regulate tumor-suppressive or tumor-promoting functions in different cell types will be important to study and is a future direction for cancer therapy.

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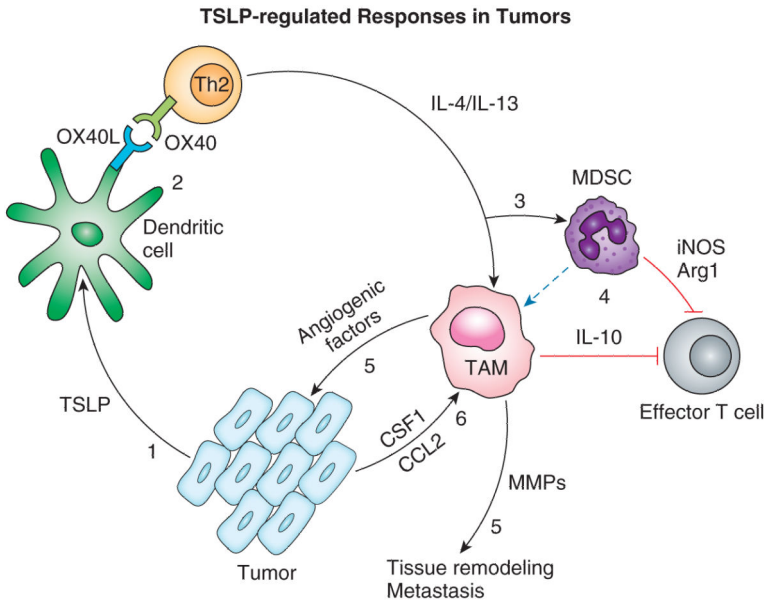

Figure 1. Schematic of TSLP-regulated responses in tumors

(1) Tumor or tumor-associated stromal cells produce TSLP, which promotes the maturation of resident dendritic cells through upregulation of costimulatory molecules, including OX40L; DCs drive the differentiation of Th2 cells through OX40/OX40L interactions (2); Th2 cells secrete IL4 and IL-13, leading to the recruitment and activation of MDSCs and tumor-associated macrophages (TAM;3), both of which are capable of effector T cell responses against the tumor (4); TAMs also produce factors that promote angiogenesis and matrix remodeling (5), while the tumor and associated stromal cells produce chemotactic and survival factors for the TAMs (6). Dotted line: proposed differentiation path.

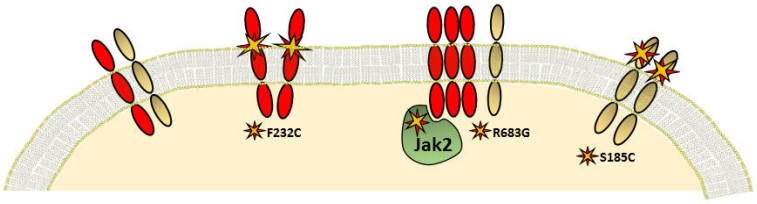

Figure 2. Schematic of TSLPR-IL7Rα mutations in B-ALL. L-R

TSLPR (red) and IL-7Rα (grey)-Wild type receptor complex; TSLPR homo-dimer with F232C mutation; Increased TSLPR expression and R683G JAK2 mutation; IL-7Rα homo-dimer with S185C mutation.