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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 nonhematopoietic cell lineages. These include dendritic cells, basophils, mast cells, B cells, epithelial cells, and CD4⁺, CD8⁺, and NK T cells. Although dendritic cells, basophils, mast cells, B cells, epithelial cells, and CD4⁺, CD8⁺, and NK T cells. Although TSLP’s role in the promotion of Th2 responses has been studied extensively 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 highlights recent advances in the understanding of the surprising role of TSLP in the control of a variety of cancers, both solid tumors and leukemia, in which the TSLP/TSLP receptor axis was shown to be an important regulator. The Journal of Immunology, 2014, 193: 4283–4288.

Thymic stromal lymphopoietin (TSLP) is a member of the four-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 costimulate 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 γ-chain (γc) (7). It is now known that the functional, high-affinity TSLP complex is a heterodimer of TSLPR and 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, despite a loss of sequence identity.

The similarity of TSLP to IL-7 and the homology of TSLPR to γc suggested that TSLP may play a role in regulating lymphocyte development and/or function. Indeed, early studies showed that TSLP was capable of influencing both T and B cell development and proliferation 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 is myeloid dendritic cells (DCs) (5). Confirming the expression data, TSLP treatment of human DCs induced several phenotypic changes, including increased survival, up-regulation of MHC class II and costimulatory 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 about the signaling pathways that are activated following engagement of the TSLP complex. Initial studies in the mouse showed that STAT5 was activated, but in the absence of detectable Jak activation (2), making TSLPR unique among members of the hematopoietic receptor family. However, two subsequent articles demonstrated robust and sustained activation of JAK-1 and JAK-2 following TSLP signaling in primary human DCs and primary human and mouse CD4⁺ T cells (5, 14). Surprisingly, unlike IL-7Rα and γc in IL-7 signaling, which use JAK-1 and JAK-3, the TSLP subunit bound and used 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 showed that, in addition to STAT5, TSLP stimulation activated STAT1, STAT3, STAT4, and STAT6, as well as JAK-1 and JAK-2 (15). One possible explanation for the discrepancy in the data between species is that the mouse-signaling study primarily used a pre-B cell line, whereas the human studies largely were performed in primary DCs. Consistent with this explanation, our laboratory showed that TSLP-treated mouse DCs activate Jak1 and Jak2, as well as STAT1, STAT3, and STAT5, although only Stat5-deficient DCs failed to induce TSLP-specific genes (16). In addition, studies using nonhematopoietic cells (airway smooth muscle cells) showed that TSLP signals through Stat3 (17). Taken together, these data demonstrate that TSLP is capable of activating multiple STAT proteins. Whether TSLP uses similar
signaling pathways in other cell lineages and how each STAT molecule contributes have yet to be elucidated.

As mentioned above, several cell types were shown to respond to TSLP. Originally, TSLP was isolated and characterized as a lymphocyte growth factor (1, 2, 4), and subsequent studies showed that it 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 is myeloid-derived DCs (5, 13). Coculture of TSLP-activated DCs with naive 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 Ag-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 TNF-α), 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 OX40 ligand (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 also were 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 suggested a role for TSLP in the generation of tolerogenic DCs that can drive the differentiation of regulatory T cells (26–28), although other studies indicated that TSLP may hinder the production and/or maintenance of FOXP3+ regulatory T cells in vivo in certain disease processes (29).

Finally, several innate immune cells express TSLPR and respond to TSLP. For example, TSLP can enhance cytokine production from mast cells, NKT cells, and eosinophils (30–32). Recent work 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 (Fig. 1). Although 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 was found for the induction and regulation of a variety of tumors. TSLP was found to both promote and suppress solid tumor growth, and somatic mutations and chromosomal translocations in genes encoding members of the TSLPR complex have been found in a subset of pediatric patients with B cell acute lymphocytic leukemia (B-ALL). The remainder of this review discusses that 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 was shown that, for many types of cancers, a Th2 response is dominant over cytotoxicity induced by CD8 T cells and a Th1 response (34). Tumors with this type of phenotype generally have a worse prognosis relative to tumors in which 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 in which a GATA3+ Th2 cellular infiltrate is dominant, showed that cancer-associated fibroblasts can produce TSLP. In vitro, supernatants from cancer-associated fibroblasts were capable of activating DCs to drive Th2 differentiation. Importantly, they found that tumors and tumor-draining lymph nodes contained TSLPR+ DCs, whereas nondraining lymph nodes

![FIGURE 1. Schematic diagram of TSLP-regulated responses in tumors.](Image)
did not (36). Finally, using a completely in vitro system, TSLP was shown to be released by human cervical carcinoma cells (37). These investigators suggested that this tumor-derived TSLP can act on TSLP-R+ endothelial cells to promote angiogenesis in cervical cancer. These data suggest that there is cross-talk between hematopoietic 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 CD4+ T cells to differentiate into naive CD4+ T cells. In addition, OX40L+ DCs were found in breast tumors. Interestingly, they used a xeno transfer model to show that deletion of TSLP in wild-type mice inhibited tumor formation. 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 Ph-like patients had chromosomal rearrangements involving the TSLP gene (49, 50). These Ph-like rearrangements include deletions that join TSLP and P2RY8 (a gene closely linked to TSLP) and translocations between TSLP and the IgH locus (51–53). These alterations lead to increased expression of the TSLP by coupling its expression to the promoter/enhancer of the translocation partner. In addition, these translocations were seen in ~60% of acute lymphoblastic leukemia cases in children with Down 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 TSLP gene expression are associated with a form of B-ALL with poor prognosis.

In contrast to these studies that suggest a tumor-promoting role for TSLP, two independent groups demonstrated a tumor-suppressing role for TSLP in a murine 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 TSLP-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 laboratory 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 (E.L. Kuan and S.F. Ziegler, manuscript in preparation). Although the underlying mechanism is not yet clear, we found that TSLP can functionally activate monocyte lineage myeloid-derived suppressor cells and that they are lacking in the tumor-bearing TSLP-deficient hosts.

In contrast to these studies that suggest a tumor-promoting role for TSLP, two independent groups demonstrated a tumor-suppressing role for TSLP in a murine model of skin cancer (41, 42). Both studies used keratinocyte-specific ablation of Notch signaling, which was shown to lead to skin barrier defects and TSLP-dependent dermatitis (43). Demehri et al. (42) showed that mice with inactivation of Notch signaling through deletion of RBp7 in keratinocytes failed to develop skin tumors, even following a chemical carcinogenesis regimen that led 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 that 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 that TSLPR signaling may function differently in distinct cell types. 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 study by Demehri et al. (42) is that the timing and magnitude of TSLP expression are critical. Interestingly, a recent study using the 4T1 transplant breast tumor model showed that transplanted TSLP-deficient mice displayed greater metastasis to the brain but lesser metastasis to the lung (45). They claimed that this paradoxical result may occur because the blood–brain barrier keeps tumor cells out, but the enhanced systemic Th1 responses that they observed in TSLP-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 nontumor normal donors. TSLPR complex and pediatric B-ALL

Pediatric acute lymphocytic leukemia 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; although 80% of affected children are treated successfully, it remains a leading cause of childhood morbidity and mortality (47, 48). Recent advances in molecular genetic profiling of B-ALL uncovered the origin 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; however, they are also seen in Ph− cases that resemble Ph+ patients (referred to as Ph-like acute lymphocytic leukemia) (49, 50). These Ph-like cases account for ~15% of B-ALL, and patients have a higher risk for relapse compared with Ph+ patients.

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 Ph-like patients had chromosomal rearrangements involving the TSLP gene (49, 50). These Ph-like rearrangements include deletions that join TSLP and P2RY8 (a gene closely linked to TSLP) and translocations between TSLP and the IgH locus (51–53). These alterations lead to increased expression of the TSLP by coupling its expression to the promoter/enhancer of the translocation partner. In addition, these translocations were seen in ~60% of acute lymphoblastic leukemia cases in children with Down 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 TSLP 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 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 because the resulting TSLPR is able to constitutively homodimerize and signal. Interestingly, a similar mutation in IL-7Rα, which forms a heterodimer with TSLPR to generate a functional TSLPR complex, was found in B-ALL (S185C) (59). In addition, insertions and deletions within the transmembrane domain of 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 was associated with elevated TSLPR expression in B-ALL (60). These data make a compelling case for enhanced TSLPR 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 is 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). Although these experiments provided insights into the consequences of these mutations on signaling from the receptor, they did not address the nature of the cells 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 retrovirus expressing TSLPR/CRLF2 with the mutation, followed by transplantation into lethally irradiated hosts. One animal displayed splenomegaly and indications of increased myeloproliferation in the blood, as well as elevated numbers of immature granulocytes and megakaryocytes in the bone marrow (58). Although this study demonstrates that TSLPR F232C is an activated allele, there are important issues with these studies. First, the investigators 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, because other studies suggested 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, because the human and mouse cytokines are species specific in their ability to bind the TSLPR complex (7). Although these studies are important in that they demonstrate altered functionality of the mutant protein, they have limitations.

Mice with systemic overexpression of TSLP may provide a model for understanding the signaling mechanisms involved. Interestingly, overexpression 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 showed expansion of B cell compartments following TSLP expression in adult mice (62). Importantly, in these studies the target of TSLP in the bone marrow was 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 y, a new role for TSLP in tumor immunology has emerged. Interestingly, these studies found a rather complicated role for TSLP, with it 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) underlying TSLP 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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**Disclosures**

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