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HIGHLIGHT

Tertiary lymphoid structures: The touchstone for tumor immunotherapy

KEY WORDS

Tertiary lymphoid structures;
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Tertiary lymphoid structures (TLS), also referred to as tertiary lymphoid organs or ectopic lymphoid structures, are organized aggregates of immune cells that develop in non-lymphoid tissues in response to certain disease processes[1]. They are typically observed in chronically inflamed tissues that are persistently exposed to antigens, such as in autoimmune diseases, chronic infections, and cancers[2]. In many tumor types, the presence and higher density of TLS are strongly correlated with improved patient prognosis[3]. Over recent years, TLS have emerged as a research hotspot in tumor immunotherapy. Their presence, degree of maturation, and functional status serve as key biomarkers for predicting responses to immunotherapy and may represent promising targets for future therapeutic interventions[4,5]. Given their pivotal role in anti-tumor immune responses, TLS are increasingly regarded as a touchstone for evaluating the efficacy of cancer immunotherapies.

A study recently published in *Nature*[6] demonstrated that IL-33-activated ILC2 cells induced the formation of TLS in pancreatic cancer, thereby enhancing anti-tumor immunity. The study found that the inflammatory alarmin IL-33 activated lymphotoxin-expressing type 2 innate lymphoid cells (ILC2s), which collaborated with LTβR $^{+}$ myeloid cells to induce TLS formation. In a pancreatic cancer model, recombinant human IL-33 amplified lymphopoietic ILC2s and TLS, thereby enhancing anti-tumor immunity. This discovery revealed a targetable IL-33−ILC2−TLS axis, providing a new strategy for cancer immunotherapy (Fig. 1).

Under normal conditions, IL-33 resides in the cell nucleus, where it inhibits gene transcription. When tissue damage or necrosis occurs, IL-33 is released extracellularly as an alarmin, activating the immune system by directly targeting immune cells that express its receptor ST2 (IL1RL1). Balachandran and colleagues previously reported in *Nature*[7] that IL-33 promotes the expansion of ILC2s and T cells in pancreatic tumors, thereby exerting anti-cancer effects. Previous studies have identified ST2 as a receptor for IL-33[8]. The canonical IL-33/ST2 signaling pathway involves IL-33 binding to ST2, enabling immune cells in the tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC) to mount anti-tumor responses. ST2 functions as the “antenna” that allows ILC2s to perceive IL-33 signals, effectively “awakening” them to perform immune functions. Amisaki et al. further demonstrated that endogenous IL-33 is required for TLS formation in cancer and inflammatory models. In mouse models of colitis and PDAC, loss of IL-33 led to markedly reduced inflammation and impaired lymphotoxin-beta receptor (LTβR)-induced TLS formation, highlighting the critical role of cytokines in TLS development. Notably, while secondary lymphoid organs (SLOs) develop during embryogenesis and early postnatal life, TLS arise in adult tissues under pathological conditions. In both SLOs and TLS, lymphotoxin (LT) produced by inducible cells binds to LTβR on stromal cells, driving chemokine and adhesion molecule expression and orchestrating lymphoid neogenesis.

Researchers used recombinant IL-33 (rIL-33) to test its role as an extracellular trigger of TLS formation. In PDAC models, rIL-33 promoted TLS maturation, increasing intra-tumoral lymphoid aggregates (early TLS) and primary follicles (intermediate TLS), while also expanding ST2^{+} ILC2s. TLS from rIL-33-treated tumors contained BCL6^{+} B cells, with intra-tumoral B cells showing clonal expansion and somatic hypermutation, indicative of germinal center activity and secondary follicle (late TLS)

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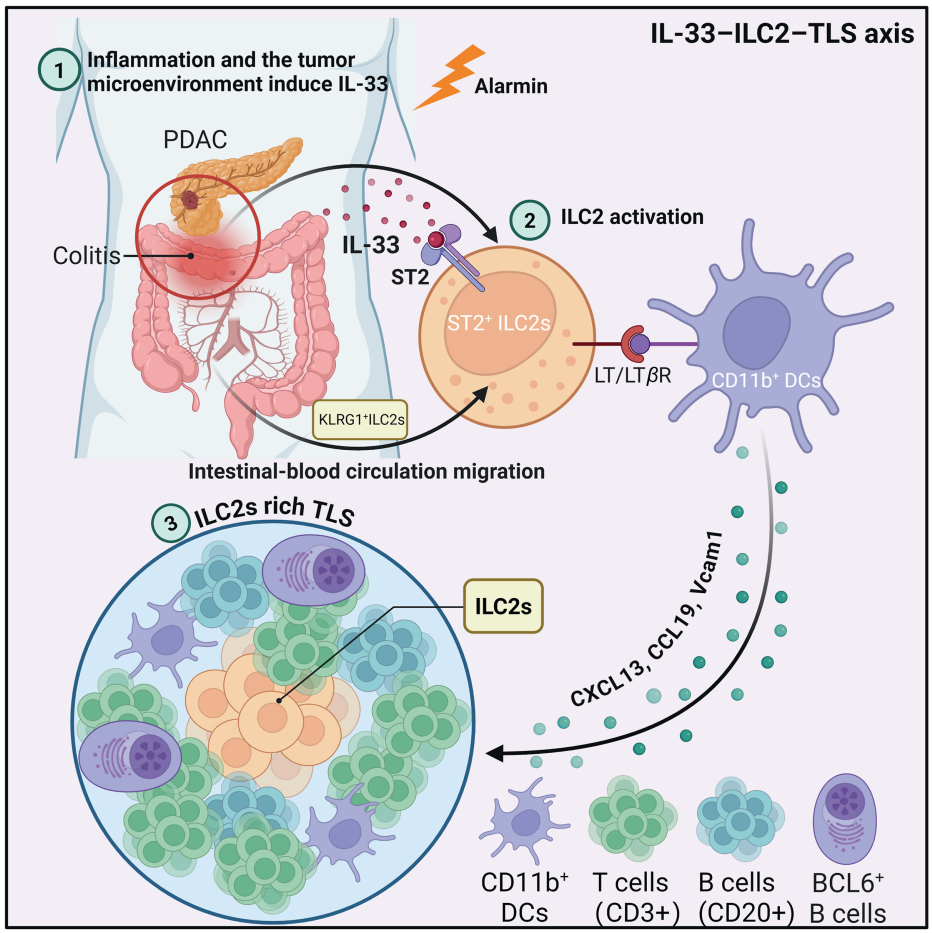

Figure 1 Mechanisms of the IL-33−ILC2−TLS axis in PDAC and colitis. Inflammatory signals in colitis and pancreatic ductal adenocarcinoma (PDAC) trigger the release of the alarmin IL-33 (①), which binds to ST2 on type 2 innate lymphoid cells (ILC2s), activating them (②). Activated ILC2s upregulate lymphotoxin (LT) and interact with LTβR $^{+}$ $CD11b^{+}$ myeloid/stromal cells to initiate tertiary lymphoid structure (TLS) formation. $KLRG1^{+}$ ILC2s proliferate and migrate from the intestinal niche to pancreatic tumors *via* the blood. Within tumors, $CD11b^{+}$ cells express high LTβR and secrete TLS-inducing chemokines (CXCL13, CCL19) and adhesion molecules (Vcam1), recruiting $CD3^{+}$ T cells, $CD20^{+}$ B cells, $BCL6^{+}$ germinal center B cells, and dendritic cells (③). This IL-33−ILC2−TLS pathway represents a novel mechanism of tumor-associated lymphoid neogenesis and a potential target for enhancing anti-tumor immunity in PDAC.

formation. TLS compartmentalization was guided by chemokine-producing stromal cells, such as $CCL19^{+}$/$CCL21^{+}$ fibroblastic reticular cells and $CXCL13^{+}$ follicular dendritic cells, which direct $CCR7^{+}$ T cells and $CXCR5^{+}$ B cells into distinct zones[9,10]. In PDAC and DSS colitis models, rIL-33 expanded $KLRG1^{+}$ ILC2s that expressed lymphotoxin (LT). Amisaki et al. showed that these ILC2s migrate from the intestine to PDAC *via* the gut−blood axis in a microbiota-dependent manner. At tumor sites, $KLRG1^{+}$ ILC2s and $CD11b^{+}$ myeloid cells acted as TLS-inducing cells and stromal organizers, respectively, cooperating to amplify IL-33 signaling and drive TLS formation. Notably, intra-tumoral LTβR $^{+}$ myeloid cells expressed higher levels of TLS-inducing chemokines (Cxcl13, Ccl19), the adhesion molecule Vcam1, and other inflammatory chemokines, thereby promoting recruitment and organization of TLS components.

The study identified $KLRG1^{+}$ ILC2s expressing lymphotoxins in human tumors, supporting their lymphopoietic role and the therapeutic potential of the IL-33−ILC2−TLS axis. Amisaki et al. engineered a recombinant human IL-33 with enhanced activity and extended half-life, which dose-dependently expanded $KLRG1^{+}$ ILC2s and TLS in tumors, underscoring its promise for PDAC immunotherapy. This work systematically demonstrated that IL-33, as a prototypical alarmin, promotes immune cell recruitment and spatial organization in the tumor microenvironment, driving TLS formation *via* the LT−LTβR pathway. Importantly, it revealed ILC2s as the key responders to IL-33, capable of proliferating and migrating from the intestine to tumors, thereby linking the gut immune environment with PDAC. Collectively, these findings establish the IL-33−ILC2−TLS axis as a central mechanism of TLS neogenesis in cancer. These findings provide a foundation for novel immunotherapeutic strategies that enhance anti-tumor immunity by promoting TLS formation and function. The study comprehensively delineates the IL-33−ILC2−TLS axis in pancreatic

cancer, highlights how inflammatory signaling shapes tumor immune responses, deepens understanding of the PDAC immune landscape, and points to new opportunities for improving patient outcomes through targeted modulation of TLS.

Limitations and future work

TLS, as "specialized immune zones" in the tumor microenvironment, represent promising biomarkers and therapeutic targets. This study highlights the importance of the IL-33−ILC2−TLS axis in pancreatic cancer, but several limitations remain. Most findings are based on mouse models, and clinical translatability and safety are yet to be established. IL-33 itself exerts dual effects, with both anti- and pro-tumor potential. The mechanisms of TLS induction remain incompletely understood, including the cellular sources, secretory signals, and migration pathways involved. Moreover, the heterogeneity and microbiome-regulated plasticity of ILC2s complicate predictions of therapeutic efficacy.

Future studies in human populations are needed to validate TLS as predictive biomarkers, develop standardized systems for assessing their quality and function, and explore strategies to combine engineered IL-33 with therapies such as immune checkpoint inhibitors. With precise delivery and safety control, targeting TLS could become a valuable approach for advancing precision immunotherapy.

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Author contributions

Lisha Zhou: Writing, review and editing; Original draft writing. Shunji Liu: Writing, review and editing; Conceptualization. Yang Sun: Review and editing; Funding acquisition and Conceptualization.

Conflicts of interest

The authors declare no competing interests.

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